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ORGAN-FORMING SUBSTANCES IN THE EGGS OF ASCIDIANS.

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WITH 24 PHOTOMICROGRAPHS OF LIVING EGGS OF CYNTHIA (STYELA) PARTITA STIMPSON.

BY KATHARINE FOOT AND E. C. STROBELL.

That the egg of any animal is composed of "simple, undifferentiated protoplasm" is an article of traditional belief with a large number of zoölogists, and that the cleavage of the egg is "a mere sundering of homogenous materials capable of any fate" is a doctrine which has been given great prominence in recent years. In favor of these commonly accepted views stands a considerable body of experimental work on the development of the ovum; fragments of eggs or isolated blastomeres in many cases are said to give rise to entire larvæ, thus proving, as is usually claimed, that the parts of the egg or embryo are still undifferentiated at the time of the experiment.

But not all experiments on development have confirmed these conclusions; some of the first and most careful researches of this sort led to directly opposite results. In the development of the frog's egg Roux found (1883, 85, 87, 89, 92, 93, 94, etc.) that the median plane of the embryo is determined in the egg immediately after fertilization and that "the development is, from the second cleavage on, a mosaic work of at least four vertical independently developing pieces." In 1887 Chabry showed that the early cleavage cells of the ascidian egg are specified for particular ends and that they develop, if they develop at all, into parts which they would produce under normal conditions. These results were, however, denied on the ground of other ex-

perimental work, and by many it was held that the "mosaic theory had received its death blow from the facts of experimental embryology." Among the ova in which isolated blastomeres were found to be capable of complete development were those of ascidians and from this fact it was assumed that here also the early cleavage cells were undifferentiated.

Nevertheless, although some of the results of experimental embryology seemed to render the mosaic theory untenable, additionable evidence in favor of it was furnished by another line of work commonly known as "cell-lineage." In a considerable number of animals belonging to distinct phyla (annelids, mollusks, polyclades, nemerteans, nematodes, rotifers, crustaceans and ascidians) the cleavage of the egg was found to be constant in form and differential in character and each of the early cleavage cells was shown to play a perfectly definite part in the building of the embryo. By those who maintained the traditional view as to the simplicity of the egg and the homogeneity of the blastomeres this fact was explained as due to "the continuity of development," "the position of the blastomeres in the cell complex," etc. But this explanation was never a satisfactory one and is no longer tenable; both observation and experiment have shown conclusively that in certain eggs the blastomeres are not all alike. In particular the experimental work of Crampton (1896), Fischel (1897, 1898), Boveri (1901), Wilson (1903, 1904), Yatsu (1904) and Zelney (1904) has demonstrated that in ctenophores, echinoderms, nemerteans, mollusks and annelids all portions of the egg are not equipotential; this, as well as other work on the organization of the egg, proves that there is a differentiation and localization of the substances of the egg very unlike the "simple undifferentiated protoplasm" of traditional belief.

In most instances the protoplasm of the different blastomeres of an egg is much the same in appearance; in a few cases it is visibly different, but in all cases which have been carefully studied definite blastomeres always give rise to definite parts of the embryo. In fact the manner and rate of development as well as its results are so thoroughly characteristic of certain blastomeres that students of cell-lineage have usually concluded that the protoplasm of different blastomeres must differ, even though these differences

are not directly visible. Recent experimental work on some of these forms confirms and extends these conclusions and proves that even in the egg before cleavage begins different substances may be present which are destined in the course of development to enter into specific parts of the embryo.

The most notable differentiations of the oöplasm which have been observed hitherto are found in *Myzostoma* (Driesch, 1896; Wheeler, 1897; Carazzi, 1904), in *Strongylocentrotus* (Boveri, 1901), in *Unio* and *Chætopterus* (Lillie, 1901, 1902), in *Dentalium* and *Patella* (Wilson, 1904) and in the gasteropods *Crepidula*, *Physa*, *Planorbis* and *Limnæa* (Conklin, 1902, 1903). In none of these cases, however, are the differentiations and localizations of the oöplasm so remarkable as are those which occur in the ascidian egg. Here the different substances of the egg are strikingly dissimilar; they are localized in their definitive positions at a remarkably early period, and they may be traced with ease and certainty through the maturation and fertilization, the cleavage, the gastrulation and the later stages until they give rise to specific organs or parts of the larva.

Material and Methods.

I have studied the early differentiations of the egg in three species of simple ascidians, viz., *Cynthia* (*Styela*) *partita* Stimpson, *Ciona intestinalis* (L.) Flemming, *Molgula manhattensis* Verrill. The differentiations and localizations are essentially the same in all of these species, but as the different kinds of oöplasm are more brilliantly colored in *Cynthia* than in either of the other genera named, I shall devote particular attention to this form.

In an extensive publication on the organization and cell-lineage of the ascidian egg (Conklin, 1905), I have figured and described the remarkable localization of germinal materials in the egg of *Cynthia*. The differentiations of the egg substance are here so great and their localization so precise that the figures and descriptions of these might well seem to be exaggerated. I therefore welcome the opportunity of publishing a series of photomicrographs of these eggs, an opportunity which I owe to the skill and courtesy of Misses Foot and Strobell. Their method of photomicrography, which they have fully described in previous

publications (Foot and Strobell, 1899, 1900, 1901, 1902) is, in some respects, the most satisfactory ever devised and yet it is as simple as it is complete. The distinguishing feature of this method consists in the accuracy and rapidity with which an exact focus can be obtained; this fact will be better appreciated when the photographs which illustrate this paper are examined in detail and when it is said that thirty such photographs, all of them satisfactory, were taken in less than four hours. With more time and material a more complete series of stages might have been photographed, but this series is sufficiently complete to show the principal features in the differentiation and localization of the egg substances.

All of the photographs are of living eggs in sea water and were taken with a Zeiss Apochromat Obj. 16 mm., Compensating Oc. 4, the bellows length being sufficient to make the magnification 112 diameters. The photographs have not been reduced in the process of reproduction and *neither the negatives nor prints were retouched or altered in any respect whatever.*

The eggs photographed were artificially fertilized and the earliest stage shown (Photo 1) was taken about three quarters of an hour after the sperm were mixed with the ova, but not more than fifteen minutes after the entrance of the spermatozoön into this particular egg. The method of procedure was to place a large drop of sea water containing a considerable number of eggs on a slide and cover with a glass supported by glass "feet" 170 μ thick (the eggs are about 150 μ in diameter). Suitable eggs were then selected and photographed by daylight, the exposure ranging from ten seconds to one minute; the shorter exposure was found to be sufficient while the longer was greatly overtimed (Photos 1 and 13). In order to obtain good color values it is necessary to have the diaphragm rather widely open and this renders the definition somewhat less distinct than it otherwise would be. Furthermore a low magnification was necessary in order to secure sufficient depth of focus to show the entire egg; even with the power employed it was not possible to bring the whole of the egg into good focus at one time. In spite of these evident disadvantages the photographs are really remarkable. Few, if any, other eggs are known in which the organization is so notable as in *Cynthia*

and certainly nothing like this has ever before been photographed in the living condition. I wish here to express my great indebtedness to Misses Foot and Strobell for their kindness in making the photographs and also in superintending the production of the plate.

Differentiations and Localizations of the Oöplasm.

In the ovarian egg of *Cynthia* there is a peripheral layer of protoplasm, free from yolk, in which the "test cells" are imbedded and which contains numerous orange-yellow pigment granules which are uniformly distributed. The central portion of the egg consists of yolk-laden protoplasm which is slate-gray in color and lying somewhat eccentrically within the egg is the large, clear germinal vesicle. Before the egg leaves the ovary the chorion is formed and the "test cells" are extruded into the space between the egg and the chorion.

After the egg is laid the germinal vesicle breaks down, but the polar bodies are not formed until after the egg is fertilized. If fertilization does not take place the polar bodies are never extruded, and the clear, the yellow and the gray substances remain in the positions in which they were before the wall of the germinal vesicle disappears. If the egg is fertilized, however, a most astonishing series of movements occur which lead to the localization of the different oöplasmic substances in definite regions of the egg.

The spermatozoön always enters the egg near the vegetal pole and immediately after its entrance the peripheral layer of yellow protoplasm flows rapidly to this pole where it collects as a cap[1] (Photos 1 and 2). At the same time the clear substance derived from the germinal vesicle also flows to the lower pole where it lies between the cap of yellow protoplasm below and the yolk above. This streaming of protoplasm to the lower pole takes place so rapidly that its movements can be directly observed. Within ten or fifteen minutes after the entrance of the sperm into the egg all of the clear and yellow protoplasm has collected at the lower pole while the opposite pole where the polar bodies

[1] In all the photographs the yellow substance appears very dark, the gray substance less dark while the clear protoplasm is relatively light.

are formed is at this stage rich in yolk and is slate-gray in color.

After the clear and the yellow substances have collected at the lower pole the sperm nucleus and aster move toward one side of the egg which future development shows to be the posterior pole; the clear and yellow substances are also drawn over to this side of the egg and in such a manner that the yellow cap is transformed into a superficial band or crescent which lies just below the equator of the egg on the posterior side, its arms extending forward on each side about half way around the egg (Photos 3 and 4). Owing, perhaps, to the way in which this crescent is formed from the cap of yellow protoplasm its ventral border is sharper and its substance is of a deeper yellow than the dorsal border. At the middle of the yellow crescent is a small area of clear protoplasm which first gathers around the sperm as it enters the egg and which afterward lies at the middle of the crescent throughout the entire development; this clear protoplasm is seen in profile at the middle of the crescent in Photo 3.

The movement of the protoplasm to the posterior pole is apparently initiated by the movement of the sperm nucleus and aster to this pole; here the sperm aster divides giving rise to the amphiaster and here the two germ nuclei meet. The axis of elongation of the amphiaster is always at right angles to the axis which connects the animal and vegetal poles while its middle lies in the plane of the first cleavage and in the median plane of the embryo. The amphiaster lies beneath the yellow crescent and some distance from the surface of the egg and the long axes of the two coincide. In Photo 4 the clear line in the middle of the crescent is the amphiaster seen through the superficial layer of the crescent. It is probable that there is some causal connection between the elongation of the amphiaster and the formation of the crescent.

The clear protoplasm which also moves to the posterior pole along with the yellow is chiefly withdrawn from the surface and aggregated around the sperm nucleus and aster, though a portion of it comes to the surface just above (ventral to) the crescent (Photo 3). As the time for the first cleavage approaches the

germ nuclei and amphiaster move to the center of the egg and the clear protoplasm goes with them. Finally in the telophase of the first cleavage the clear protoplasm moves into the upper (animal) hemisphere, while the yellow and gray protoplasm are situated in the lower (vegetal) hemisphere.

The first cleavage cuts through the middle of the crescent, the clear protoplasm, and the yolk, the division of all the egg substances being bilaterally symmetrical. *At the close of this cleavage each of these substances occupies its definitive position in the egg (Photos 6–8). The upper clear half of the egg gives rise to ectoderm; the crescent of yellow protoplasm surrounds the posterior side of the egg just below the equator and is later transformed into the muscle and mesenchyme cells of the larva; the gray protoplasm occupies the remainder of the lower hemisphere and gives rise to the endoderm, to the chorda, and to the neural-plate.* Two areas are distinguishable in the gray substance, though I had failed to see them until my attention was called to them by the photographs; the posterior part of the gray material lying in front of the crescent and extending some distance anterior to the vegetal pole is deeper in color and contains more yolk than the anterior portion; the latter forms a light gray crescent around the anterior border of the vegetal hemisphere, just as the yellow protoplasm forms the yellow crescent around its posterior border. The 2-cell stage when seen from the right or left sides (right side in Photo 8) shows all of these areas distinctly, the yellow crescent at the posterior pole (very dark in the photograph), the deep gray material anterior to this, and the light gray crescent occupying the anterior third of the vegetal hemisphere and extending a little above the equator, while the clear protoplasm is located chiefly in the upper hemisphere. The dark gray portion of the vegetal hemisphere gives rise to the endoderm of the larva, the light gray crescent to the notochord and neural plate.[1] *All the principal organs of the larva in their definitive positions and proportions are here marked out in the 2-cell stage by distinct kinds of protoplasm!*

[1] It is an interesting question whether the chorda-neural-plate crescent of the ascidian egg corresponds to the "gray crescent" of the frog's egg. In both cases it lies on the anterior side of the egg and in the vicinity of the dorsal lip of the blastopore, but it is not positively known that chorda and neural plate are derived from it in the frog, as is the case in the ascidian, though this seems probable.

Although these different oöplasmic substances are chiefly localized in certain regions of the egg, which give rise to certain portions of the embryo, this segregation is not quite complete. Most of the clear protoplasm is found in the upper (ectodermal) half of the egg but some of it is also present in the lower half. Most of the yolk is found in the lower (endodermal) half of the egg, but a little of it is found in the upper half. Almost all of the yellow protoplasm is located in the mesodermal crescent, but a very small amount of it is found around the nuclei of all the cells. Thus samples of all of these egg substances are contained in all of the cells; nevertheless the segregation is so nearly complete that the clear, the gray, the light gray and the yellow areas are marked out with the greatest distinctness (Photos 7, 8).

In the 4-cell stage, as shown by Photos 9 and 10, the distribution of these substances remains as in the 2-cell stage, the yellow crescent being confined to the vegetal hemisphere and the posterior quadrants, the gray crescent to the vegetal hemisphere and the anterior quadrants, while the deep gray, yolk-laden substance lies between these crescents at the vegetal pole and the clear protoplasm occupies most of the animal hemisphere of the egg.

In the 8-cell stage the localization of these substances is the same as in the preceding stages, the clear protoplasm lying above the third cleavage plane and the other substances below it (Photo 11). The perfectly sharp boundaries of the crescent do not coincide with any of the cell boundaries, gray substance being found in the posterior dorsal cells above, below, and anterior to the crescent (Photo 11). The clear notch in the posterior profile of the crescent in Photo 11 is a cap of the same clear protoplasm which gathered around the sperm head at its entrance and afterwards lay at the middle of the crescent (Photo 3). In the 8-cell stage this clear protoplasm takes the form of two caps on the surface of the yellow crescent and adjoining, on each side, the median plane. In this same stage a small amount of yellow protoplasm may be seen around the nuclei of all the cells (Photo 11). This perinuclear yellow substance is most abundant in the posterior-ventral and in the anterior-dorsal cells; in the former it lies chiefly on the dorsal and lateral sides of the nuclei, in the latter on the posterior and lateral sides. In subsequent divisions of these

cells this perinuclear plasm retains these positions and therefore goes into certain daughter cells and not into others. Consequently even in those cells in which it is found very sparingly this yellow protoplasm is localized with great definiteness.

In the 16-cell stage all these different substances remain in exactly the same positions which they held at the 2-, the 4- and the 8-cell stages (Photos 12–14). The eight ventral cells are all similar in appearance and are composed chiefly of clear protoplasm. The yellow crescent still surrounds the posterior half of the dorsal hemisphere, as in the 1-cell stage, but it is now contained in four cells; it occupies the posterior and lateral portions of these cells while the anterior and median portions are composed of gray substance. The boundary between these two substances could not be sharper if they were actually, as at first sight they seem to be, separated by a cell wall (Photo 14); only at the next stage, however, are these substances segregated into separate cells. The gray crescent occupies the anterior portions of the four anterior cells of the dorsal hemisphere, the posterior portions of these cells being composed of deep gray (endodermal) substance (Photo 14).

At the 32-cell stage (Photos 15, 16) the substances of the yellow and the gray crescents are finally segregated into separate cells, although a small portion of deep gray substance is still contained in the median cells of the yellow crescent; however this gray material moves in from the surface so that it does not show in photographs of the entire egg. The yellow crescent now consists of six cells, four median ones which are small and one pair of lateral ones which are relatively large (Photos 15, 16). The gray crescent consists of four cells of equal size and similar constitution. The ventral half of each cell is clear, contains little yolk and gives rise to most of the neural plate, the dorsal half is light gray in color, is yolk-laden and gives rise to the chorda. From this stage onward the ventral hemisphere is composed of clear cells, all of which are much alike; for this reason no photographs are given of the later stages of these cells.

In subsequent stages all of the cells of the gray and yellow crescents divide in a vertical direction (parallel to the egg axis); these divisions occur first in the gray crescent and in the most

anterior pair of cells of the yellow, then in the pair of yellow cells adjoining the latter behind, and finally in the posterior median pair. The subdivision of the cells of the gray crescent occurs at the 44-cell stage; the ventral product in each case is a small clear cell which ultimately forms the posterior portion of the neural plate, the dorsal products are larger and are gray in color and ultimately develop into the chorda.

In the case of the yellow crescent both products of the posterior median cells give rise to mesenchyme, but the ventral ones contain those caps of clear protoplasm which were visible in the 8-cell stage (Photo 11) and which in a still earlier stage first appeared around the entering spermatozoön (Photo 3). The dorsal products of the other two pairs of crescent cells give rise to mesenchyme, while from the ventral halves come the muscle cells of the tadpole's tail. The mesenchyme cells are clear and faintly yellow in color, the muscle cells are a deep yellow and these two substances are distinguishable in their definitive positions even as early as the 1-cell stage (Photo 3).

Unfortunately no photographs of the early stages of gastrulation were taken. I have however studied and drawn every step of this process both in living and in prepared material. Gastrulation begins with the depression of the endoderm cells just posterior to the chorda cells, and is later continued by the rolling in of cells around the margin of the blastopore. In this manner the neural plate cells come to overlie the chorda cells, and the muscle cells, the mesenchyme.

In the closure of the blastopore the posterior (ventral) lip remains stationary until the last stages of the process, while the anterior (dorsal) lip grows backward over the gastrocoel until the blastopore is reduced to a longitudinal groove between the muscle cells of each side (Photo 17). In the overgrowth of the dorsal lip the rows of muscle cells as well as the blastopore groove are forced to the hinder end of the embryo and the muscle rows are tilted up at their anterior ends until they are transverse to the long axis (Photo 17). Later the ventral lip overgrows the remnant of the blastopore and the ectoderm of this lip forms a pair of V-shaped folds which fuse from behind forward and thus cover the dorsal lip and roll the neural plate up into a tube. In

this overgrowth of the ventral lip the transverse rows of muscle cells again assume an antero-posterior direction in the embryo (Photos 18–21).

Since the neural plate is composed of relatively transparent cells which overlie the gray chorda plate, it is not well shown in the photographs, unless seen in profile (Photos 22, 23). In the dorsal view shown in Photo 18 seven or eight transverse rows of cells may be indistinctly seen in the neural plate. The chorda plate, which contains considerable yolk and is gray in color lies under the neural plate. It consists at first of a single transverse row of eight cells, then by the division of these cells two such rows are formed and finally by shoving together from the sides this plate becomes much narrower and longer. In later stages the chorda plate and the neural plate push back between the muscle cells of each side until they reach the hinder end of the embryo, thus establishing the characteristic appearance of the young larva shown in Photo 21. It can now be seen distinctly that the deep yellow cells have become the lateral muscles in the tail, that the light gray cells of the chorda plate have formed the fusiform chorda which lies between the muscle cells, and that the deep gray cells form the gastral endoderm. At the posterior end of the chorda is a group of light yellow cells which connect the muscle rows of the right and left sides; these are the caudal mesenchyme cells. At the anterior ends of the muscle rows are the clear areas of the trunk mesenchyme, which are also of a faint yellow color, while around the entire embryo is a clear layer of ectoderm cells (Photo 21).

The form of the larva is now well established and subsequent development changes this form only in minor features. In Photo 23 the tail is much elongated and bent toward the ventral side. Three rows of muscle cells with clear nuclei can be seen on the left side of the tail, while the larva is tilted toward the left so that the dorsal row of muscle cells of the right side is also visible; with the elongation of the tail the individual muscle cells have become much longer than in previous stages. Between the dorsal rows of muscle cells of the right and left sides is a clear line which is the neural tube; anteriorly this tube overlies the dark gray endoderm and hence it is not clearly visible in the

photograph, but its anterior end appears as a clear, triangular area, notched where the tube is still open at the anterior end of the larva. This clear, transparent condition of the nervous system, both in the tail and trunk regions shows that the yellow protoplasm does not enter into its formation and that the muscle cells are not "neuro-muscular" cells as claimed by Castle (1896). In this and the following photograph (Photo 24) the larva is well developed, though the sense organs have not yet appeared in the sense vesicle. The most important organs of the larva are here clearly recognizable in the photographs of the living tadpoles, viz., the muscles, the notochord, the central nervous system, the gastral endoderm, the caudal and trunk mesenchyme and the ectoderm. *The substance of each of these organs is peculiar in color and constitution and these different substances may all be traced back to the 2-cell stage, where they occupy positions corresponding to their ultimate locations in the larva, while the substances of the ectoderm mesoderm and endoderm are recognizable in the unsegmented egg.* With the exception of the early gastrula stages, which were not photographed, every important step in the transformation of these substances into the organs named can be followed in the photographs of the living eggs and embryos!

Nature and Potency of the Oöplasmic Substances.

The fact that definite blastomeres of the ascidian egg give rise to definite portions of the larva has long been known (Van Beneden and Julin, 1884; Castle, 1896). Furthermore Chabry (1887) found that when certain blastomeres were killed the remaining ones gave rise only to a partial larva. On the other hand, Driesch (1895, 1903) and Crampton (1897) found that individual blastomeres of the ascidian egg developed into entire larvæ. The mere observation of the egg of *Cynthia* shows that certain areas are marked out from the time of fertilization, or even earlier, by distinct kinds of protoplasm and that these areas give rise in the course of normal development to definite organs. But, in view of the work of Driesch and Crampton, by what right are these areas called organ-forming regions and what is the justification for calling the substances of these areas organ-

forming substances? The answer to both of these questions is the same, viz., in the absence of a region or substance, the organ to which it would normally give rise is not produced; and conversely each substance develops, if it develops at all, into the parts which it would normally produce. Experiments which I have carried out on ascidian eggs[1] show that the development of isolated blastomeres is strictly partial, as was first shown by Chabry and afterwards denied by Driesch. As yet I have been unable to get the isolated substances of the unsegmented egg to develop at all, but when they are isolated during the cleavage stages they develop only into the parts which they would normally produce, while the portions of the egg or embryo which lack these substances develop into embryos which lack the corresponding organs. Since the first cleavage of the egg is bilaterally symmetrical and divides all the substances of the egg equally, each of the first two blastomeres contains one half of all the organ-forming regions and substances; and since isolated blastomeres of the ascidian egg always produce rounded masses of cells which tend to close over the injured surface, many of these half embryos have the appearance of whole embryos of half size; but a careful study of living material as well as of stained preparations and sections shows that the larvæ are still incomplete up to the time of the metamorphosis. When the division of the egg or embryo is made along any other plane than the median one nothing even remotely resembling a normal larva is obtained. Every substance of the egg develops, if it develops at all, into the organs which it would normally produce, and while it has not been possible to isolate these substances in the unsegmented egg, their appearance is the same before and after cleavage begins and under these circumstances there is small room for doubting that even in the unsegmented egg these are actually organ-forming substances.

Therefore in the unsegmented egg and early cleavage stages of *Cynthia partita* we have the most complete differentiation and localization of the oöplasm ever yet reported for any egg. Apart from the nuclei, the centrosomes and the asters, there are visible in the 2-cell stage six different kinds of cytoplasmic substance,

[1] These experiments will be published in full elsewhere.

each of which gives rise to some specific portion of the larva and is here present in its definitive position and proportions, viz., the clear protoplasm which gives rise to the ectoderm, the gray substance which produces endoderm, the deep yellow substance which develops into the muscle cells, the light yellow which goes into the mesenchyme, the clear protoplasm at the middle of the yellow crescent which becomes caudal mesenchyme and the light gray substance of the gray crescent which gives rise to chorda and neural plate. Inasmuch as it is difficult to refer to these different substances of the egg by the purely descriptive terms which have been employed thus far, I propose to designate them by names suggestive of the parts to which they ultimately give rise, viz., ectoplasm, endoplasm, myoplasm, chymoplasm, caudal chymoplasm and chorda-neuroplasm.[1] Of all of these substances the mesoplasm (myoplasm and chymoplasm) alone takes its definitive position before the first cleavage; the other substances reach their final positions only at the close of this cleavage. But although the localization is not complete in the unsegmented egg the ectoplasm and endoplasm are nevertheless clearly differentiated before cleavage begins; I am unable to say whether the chorda-neuroplasm is also differentiated at this stage.

Cleavage and Germinal Localization.

In the early stages of development it is apparent that the cleavage planes do not closely follow the lines of separation between the different substances of the egg. The yellow crescent is bisected at the first cleavage; the second cleavage passes anterior to it; the third cleavage plane lies some distance above (ventral to) the upper border of the crescent; the fourth cleavage bisects the halves of the crescent on each side of the median plane. No one of these first four cleavage planes follows any one of the boundaries of the crescent; the same is also true of all the other oöplasmic substances. Although the localization of these substances is precise and definite, the localization pattern does not correspond to the early cleavage pattern. In the later cleavages some of the division walls do closely correspond with the planes

[1] The substances of the chorda and neural plate are not clearly distinguishable from each other before the 8-cell or 16-cell stage.

of separation between these substances so that these various substances are ultimately segregated into definite cells, but this perfectly definite type of localization arises without reference to cell division and is not appreciably altered by subsequent divisions. During the first cleavage the yellow crescent substance may be seen to be undergoing complex vortical movements, but this does not permanently change the form or position of the crescent. In the unsegmented egg and in all the subsequent stages of the cleavage the yellow crescent occupies its initial position on the posterior side of the egg below the equator, irrespective of the position of the cleavage planes. Likewise the gray crescent, the ectoplasm and the endoplasm occupy the same positions in the egg at the beginning of gastrulation as in the 2-cell stage; in fact so far as localization is concerned the condition at the close of cleavage is the same as at its beginning.

Cytoplasmic and Nuclear Organization.

All of these different organ-forming substances are present, and are shown in the photographs, as early as the close of the first cleavage, some of them much earlier. In fact the clear ectoplasm, the gray endoplasm and the yellow mesoplasm are recognizable in the ovarian egg. Here the mesoplasm forms a peripheral layer around the whole egg in which the "test cells" are imbedded, the ectoplasm is contained within the large germinal vesicle, while the endoplasm occupies the remainder of the cell. Tracing these differentiations still further back it is found that at least a portion of the mesoplasm comes from the sphere substance (archoplasm), which is probably derived in part from the nucleus of the last oögonic division (Conklin, 1902). The yolk also is formed by the activity of the "yolk matrix" (Crampton, 1899) or yolk nucleus which is probably derived from the sphere substance. Portions of the ectoplasm, mesoplasm and endoplasm[1] are thus

[1] It may of course be objected that the yellow, the gray, and the clear substances of the ovarian egg have not been proven to be differentiated for particular ends and this I freely grant to be the case. Furthermore I do not see how this question could be tested experimentally, especially in the case of the immature ovarian egg. The fact that these substances are visibly different from one another in the oöcyte and that in all respects they resemble the ectoplasm, mesoplasm and endoplasm of the cleavage stages, to which they ultimately give rise, is the only reason for continuing to call them by these names in this earlier stage.

derived from the nucleus, the first from the nucleus of the oöcyte, the last two from the nucleus at the last oögonic division.

This remarkable condition in which considerable portions of the oöplasm can be traced back to the nucleus is of the greatest theoretical importance. From all sides the evidence has accumulated that the chromosomes are the principal seat of the inheritance material until now this theory practically amounts to a demonstration. On the other hand all persons who have much studied cell-lineage have been impressed with the fact that polarity, symmetry, differentiation and localization are first visible in the cytoplasm and that the positions and proportions of embryonic parts are dependent upon the location and size of certain blastomeres or cytoplasmic areas. However in the fact that large quantities of "nuclear sap" containing dissolved oxychromatin escape into the cell body at every mitosis (*v.* Conklin, 1902) and that these nuclear substances then contribute to the formation of specific organ-forming substances of the cytoplasm we see a possible means of harmonizing the facts of cytoplasmic organization with the nuclear inheritance theory.

Types of Germinal Organization.

By those who maintain the view that the egg is typically composed of "simple undifferentiated protoplasm" the remarkable organization of the ascidian egg will probably be regarded as an extreme case of precocious differentiation. This may perhaps be the case but the fact that germinal differentiations and localizations occur in the eggs of annelids, mollusks, nemerteans, echinoderms, ctenophores, nematodes and ascidians shows that it is by no means a rare phenomenon and it really seems as if the burden of proof were shifted to those who maintain that the egg is typically undifferentiated. Unquestionably the egg is less highly differentiated than the embryo or larva, as organ-forming substances are simpler than the organs to which they give rise, but the evidence drawn both from observation and experiment shows conclusively that in a large number of animals the substances of the egg are not homogeneous nor equipotential. But even granting that there are cases in which there is no such differentiation of the oöplasm, this supposed lack of differentiation can apply only to

portions of the egg, the cytoplasm for instance, for of course there must be determinative factors ("determinants") somewhere in the ovum, probably in the nucleus which differ from one another in kind.

However in the phyla named the localization of morphogenic substances in the cytoplasm is sufficiently definite to warrant a comparison of one group with another. In all the ascidians which I have studied and apparently in all which have been studied hitherto, the type of localization is the same. Furthermore there is good reason for supposing that this type is essentially like that of *Amphioxus* and Amphibia (on this subject see Conklin, 1905). Judging from the work which has been done on the organization of the egg in other phyla, this chordate type is very distinct from that of annelids, mollusks, nemerteans, echinoderms, nematodes or ctenophores. In fact it seems necessary to recognize several distinct types of localization.

If one has regard only to the localization of the substances of the germinal layers there is considerable uniformity among most metazoa in their pregastrular stages. In almost all cases the ectodermal substances are localized in that hemisphere of the egg which is nearest the polar bodies, and in this the ascidians are no exception to the rule; in many cases the mesodermal substances are at first localized at the opposite pole, though only among the echinoderms (*Strongylocentrotus*, Boveri, 1901) is this localization persistent; among annelids, mollusks, and ascidians the mesodermal substances early move from this pole to the posterior side of the egg.

However in the localization of specific organ bases there are many notable differences among these phyla. In this regard the annelids and mollusks and perhaps the nemerteans belong to one type, the chordates, nematodes and ctenophores to entirely different types; in fact the localization of organ bases in the ascidian egg does not resemble that in the other phyla named any more closely than does the localization of the larval or adult organs of these phyla; indeed, the principal chordate features are already represented in the ascidian egg by characteristically localized organ bases as early as the 2-cell stage.

Since the time of Cuvier the principal criterion of homology

has been, in the words of Owen, "correspondence in the relative position and connexion of parts." Such correspondence has been found to be much more fundamental than resemblances in size, proportions, or details of structure. Similarly in germinal organization it seems probable that the relative positions and connections of organ bases are essentially alike in different members of a phylum, though in other respects, they may vary widely. A peculiar type of localization of organ bases is thoroughly characteristic of the ascidian egg and probably the same thing is true of other phyla. If this be true, different phyla do not approach one another more closely in the earliest stages of germinal localization than in the cleavage or gastrular stages.

Origin and Evolution of Germinal Organization.

The fact that there are various types of germinal localization corresponding to different types of adult organization will be explained by most persons as due to the gradual "acceleration" of development, or the shifting of adult characters back to earlier and earlier stages of the ontogeny. It is but natural that those whose attention is focused upon adult structures should regard the adult as primary, the germ as secondary; but it is surprising that embryologists also have almost universally held a similar view. Even students of cell-lineage and of the organization of the egg have generally regarded this organization as secondary and have explained it as the result of "precocious segregation," or of the "reflection of larval or adult characters back upon the egg."

Such conclusions are not founded upon observation nor experiment but upon preconceived notions as to the importance of the adult and the extreme simplicity of the germ. The whole life cycle is commonly viewed from the standpoint of the adult, and all other stages are supposed to exist *for the purpose* of leading up to a definite end stage. Similarly evolution is looked upon as the transmutation of definite end stages into others by direct modification of certain adult structures, which in some way or other modify the germ and thus become inherited.

But what is the evidence that, in either ontogeny or phylogeny, the adult is primary and the germ secondary? What is the

ground for supposing that evolutionary changes first occur in the end stages and only later affect the earlier stages in the life cycle? In spite of the age-long controversy as to the inheritance or non-inheritance of acquired characters there is no satisfactory evidence that particular modifications of any adult part ever produce specific modifications of the germ. All the evidence available seems to show that the soma stands to the germ in the relation of environment and that the only influence exercised by the former upon the latter is of a general character, as Weismann has so ably argued.[1] Furthermore the difficulty of conceiving of any method by which adult characters might be transferred to the germ is well known. No hypothesis ever yet proposed for the solution of this problem harmonizes with the established facts of oögenesis and spermatogenesis. If such transfer occurs, of which there is no sufficient evidence, it can only take place by methods of which we are at present wholly ignorant.

On the other hand there is much to be said in favor of the view that the germ is primary, the adult secondary and that heritable modifications first arise in the germ and only later appear in the adult. Apart from the fact that the germ gives rise to the adult and to other germs, it is known that in certain cases apparently slight modifications of the germ may produce profound modifications of the adult, whereas the reverse is not known to be true. One of the most convincing evidences of the truth of this view is found in cases of cross breeding, particularly in hybridization, where it is certain that hybrid characters of an offspring are directly due to the hybrid character of the germ, since they can have no other possible cause. The evidence drawn from experiments on eggs and embryos on first thought seems to be conflicting; in some cases fragments of eggs or embryos give rise only to partial larvæ and injured eggs produce only embryos showing more or less serious defects; in other cases entire embryos are produced under these conditions, but these results cannot be regarded as destructive of this argument for, as has long been maintained by Roux, such cases of entire

[1] Undoubtedly one important cause of germinal variation is to be found in the influence of changing environment upon the germ, but this is far from saying that particular modifications of the adult are transmitted to the germ.

development of egg fragments may be the result of regenerative, or regulative, processes. It is not usually possible to connect definite modifications of the adult with definite alterations of the germ from which it developed, but one remarkable instance in which this is possible is found in cases of inverse symmetry. In sinistral gasteropods, and presumably in all other cases of inverse symmetry, the cause of inversion is to be found in the inverse organization of the unsegmented egg and I have elsewhere (1903) shown reason for believing that this may be due to the maturation of the egg at opposite poles in dextral and sinistral forms. Here one of the most sudden and profound alterations of structure with which we are acquainted may be traced back to a specific modification of the germ.

These facts point to the conclusion that the complex organization of an egg, such as that of an ascidian, has not arisen through the "reflection of adult characters upon the egg," but rather that this organization is primary. Furthermore they seem to indicate that evolution has taken place, not through modifications of adult structure, but through changes in germinal organization; modifications of this organization, however produced, are probably the real causes of evolution.

This conclusion, which has grown out of a study of the complex organization of the germ and its relation to adult organization, harmonizes entirely with the mutation theory of DeVries; it indicates how mutations in elementary germinal characters may appear as widespread modifications in the mature organism; it offers an explanation of otherwise inexplicable variations of adult structure, such as inverse symmetry; and finally it suggests a possible solution of that vexed problem of the origin of phyla, not by the transmutation of one adult form into another, as is assumed in all previous hypotheses, but by relatively simple alterations of the type of germinal organization.

UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA,
December 30, 1904.

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DESCRIPTION OF PHOTOGRAPHS. (PLATE XI.)

All the photographs are of the living eggs and embryos of *Cynthia* (*Styela*) *partita* in sea water and are magnified 112 diameters. Prints from the original negatives were arranged in order and photographed and the plate is an actual print (Rotograph Process) from this negative. These photographs are not diagrams but like the specimens themselves they require and will repay careful study. That portion of the egg which appears darkest in the photographs is the orange-yellow mesoplasm, that which is lightest is the transparent ectoplasm, while that which is apparently intermediate in shade between these two is the slate-gray endoplasm. The contrast between these substances is therefore greater in reality than appears in the photographs. Every egg or embryo is inclosed in a transparent chorion which does not show in the photographs; within the chorion and around the periphery of the egg are numerous "test cells" which contain yellow pigment.

PHOTO 1. Egg about fifteen minutes after the entrance of the sperm showing the yellow protoplasm as a dark cap at the lower pole where the sperm lies. The clear protoplasm is the light zone above the yellow; the rest of the egg is gray.

PHOTO 2. Egg about twenty minutes after the entrance of the sperm; the egg substances are shown as in the preceding photograph.

PHOTO 3. Egg about thirty minutes after the entrance of the sperm, seen from the right side. The yellow protoplasm is moving to the posterior pole and forming the crescent there; the clear protoplasm lies chiefly above (ventral to) the crescent; in the middle of the crescent and at the periphery of the egg is a small spot of clear protoplasm (caudal chymoplasm) which first appears around the entering sperm and ultimately goes into a pair of caudal mesenchyme cells.

PHOTO 4. Egg about thirty-five minutes after the entrance of the sperm, showing the yellow crescent as a dark band with a clear area through its center; the latter is the first cleavage spindle. Both the crescent and the periphery of the egg show a slight notch in the lower (ventral) border, which is the beginning of the first cleavage furrow. The egg is somewhat obscured by overlying test-cells which here and elsewhere give it a mottled appearance; high focus.

PHOTO 5. Egg about forty minutes after the entrance of the sperm, viewed from the posterior ventral pole. The cleavage furrow is deepest in the region of the crescent. Above the crescent is the clear (ventral) ectoplasm. High focus.

PHOTO 6. Egg about forty-five minutes after the entrance of the sperm. Two-cell stage viewed from the animal pole showing the yellow crescent at the posterior margin of the egg.

PHOTO 7. Stage similar to the preceding, viewed from the posterior pole; below the crescent is shown the gray endoplasm of the vetegal pole, above the crescent the clear ectoplasm of the animal hemisphere, in the furrow at the middle of the crescent a small amount of clear chymoplasm.

PHOTO 8. Stage similar to the preceding, viewed from the right side (an end view of one of two cells), showing the clear ectoplasm in the upper (animal) hemisphere, the yellow crescent (mesoplasm) at the posterior pole, the light gray crescent (chorda-neuroplasm) at the anterior pole and the dark gray endoplasm between the two crescents at the lower pole. The definitive localization of these substance is complete at this stage.

PHOTO 9. Four-cell stage from vegetal pole, showing the yellow crescent across the two posterior cells. The anterior cells lie at a lower level than the posterior ones and the focus is such that only the ends of the crescent show clearly.

PHOTO 10. Four-cell stage from the animal pole, high focus; many test cells cover the egg; the yellow crescent, which lies on the lower side of the posterior cells, shows indistinctly through the egg; an area of clear protoplasm is shown in each of the cells.

PHOTO 11. Eight-cell stage from the right side; the upper cells contain the clear ectoplasm, though a small amount of yolk is found at the periphery of each cell; most of the yellow protoplasm is contained in the yellow crescent, the outline of which is very distinct, but a small amount of yellow protoplasm is found around the nuclei of all the cells; in the posterior ventral (upper) cells this lies on the lateral and dorsal side of the nucleus, in the anterior dorsal (lower) cells it lies on the lateral and posterior side of the nucleus. At the middle of the yellow crescent and seen as a notch in its posterior outline is a small cap of caudal chymoplasm (the same as that seen in Photo 3). The yellow crescent is bounded by dark gray endoplasm which extends forward to the middle of the anterior-dorsal cells; the gray crescent of chorda-neuroplasm occupies the anterior portions of these cells. The forward slant of the vertical (second cleavage) furrow and the "cross furrow" formed by it and the third cleavage are clearly shown. Photos 3, 8, and 11 are all viewed from the right side and the localizations of the same organ-forming substances in the 1-, 2- and 8 cell stages are clearly shown in these photos.

PHOTO 12. Sixteen-cell stage from dorsal side, view slightly oblique. The eight dorsal cells are clearly shown, while three transparent ventral cells are indistinctly shown on the left-anterior periphery. The yellow crescent is contained in the four posterior cells, the lighter margins of the four anterior cells represent the gray crescent. The median cells behind are nearly filled with yellow mesoplasm, save for the clear nuclei and a small wedge of gray substance in the anterior portion of these cells; the lateral portions of the cells just anterior to these are composed of yellow substance, their median portions of gray material.

PHOTO 13. Sixteen-cell stage from the posterior pole, showing the four yellow crescent cells with clear nuclei; below them is the gray endoplasm, above them the clear ectoplasm; the ectoderm cells are indistinctly shown with a trace of yellow substance around nuclei of the four posterior cells.

PHOTO 14. Sixteen-cell stage transitional to 32-cell stage, dorsal view showing eight cells. The localization of the different oöplasmic substances is the same as in Photo 12, but the focus is a little deeper. The yellow and gray crescents are remarkably distinct; between the two is the area of deep gray endoplasm; the light area on the inner border of the yellow crescent is chymoplasm.

PHOTO 15. Thirty-two cell stage, dorsal view. The small posterior crescent cells have divided transversely, forming four small cells; the large mixed cell anterior to these is just cutting off its outer yellow portion from its inner gray one. The four anterior cells have divided in an antero-posterior direction thus separating the gray crescent of chorda-neuroplasm from the endoplasm. The nuclei in all the cells appear as clear areas.

PHOTO 16. Thirty-two cell stage, dorsal view, similar to the preceding.

PHOTO 17. Advanced gastrula, posterior view, superficial focus. The blastopore groove is a narrow slit bounded on each side by four large muscle cells, which are derived from the yellow crescent; the lighter colored cells at the bottom of the groove are mesenchyme and are derived from the middle part of the crescent. The dorsal lip of the blastopore (not clearly shown in the photo) closes the groove dorsally and anteriorly.

PHOTO 18. Late gastrula, dorsal view, superficial focus. The U-shaped group of mesoderm cells (Photo 17) is seen from the open end of the U; the ectoderm overgrowing the mesoderm is seen as a light area posterior to the yellow cells; the light wedge-shaped area in the mid-line is the beginning of the neural groove; the apex of the wedge lies between the limbs of the U and marks the point at which the blastopore closes and also the posterior limit of the neural plate. The ectoderm can be seen as a zone of clear cells with transparent nuclei around the periphery and faint indications of these cells with their clear nuclei can be seen forming seven or eight transverse rows of cells across the embryo anterior to the blastopore (the neural plate). The gray endoderm seen through these ectoderm cells gives a dark appearance to all the embryo save the periphery.

PHOTO 19. Late gastrula, dorsal view, deep focus. This stage is later than the preceding and the embryo is tilted slightly toward the right side, so that the plane of symmetry is a little to the right of the middle of the photo; the mesoderm cells are no longer transverse to the long axis but are extending in an antero-posterior direction. In front of the mesoderm is a dark area, the endoderm, in which four transverse rows of cells may be indistinctly seen; around the entire periphery is the clear ectoderm. The anterior portion of the embryo is wider and the posterior part narrower than at any previous stage.

PHOTO 20. Young tadpole, ventral view, superficial focus; the larva is slightly tilted to the right so that the ventral mid-line lies to the right of the middle of the photo. Three rows of rounded muscle cells (six or seven cells in a row), with clear nuclei, lie on each side of the mid line. In front of the muscle cells on each side is a clear area of mesenchyme. The strand of caudal endoderm cells shows in the mid-line between the muscle rows of each side; anterior to the muscle and mesenchyme is the gastral endodern.

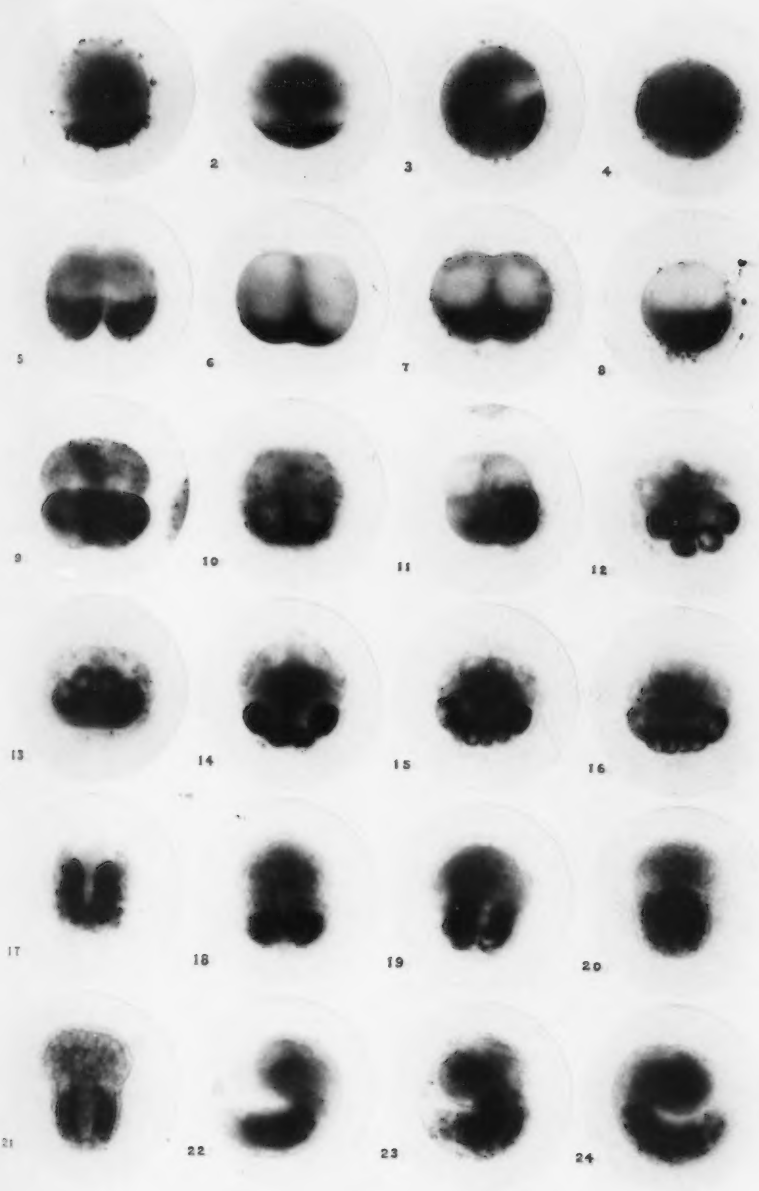
PHOTO 21. Young tadpole, ventral view, deep focus. Between the muscle cells is the fusiform notocord which is composed of wedge-shaped cells. Six muscle cells are visible on each side of the chorda and there are several lighter colored mesenchyme cells at its posterior end. At the anterior ends of the muscle rows is a clear area of mesenchyme cells in which the peribranchial pouches appear. Cells of the gastral endoderm are clearly visible; around the entire periphery are clear ectoderm cells.

PHOTO 22. Young tadpole from left side. The three rows of muscle cells, each with a clear nucleus are faintly shown in the dark area in the tail, the gastral endoderm also appears as a dark area in the trunk, but not so dark as the muscle cells. Around the entire periphery is the clear ectoderm; on the dorsal (convex) side this is especially thick and comprises the neural tube. The hazy areas ventral to the embryo are due to aggregated test cells.

PHOTO 23. Tadpole of about the same stage as the preceding, left-dorsal aspect.

The rows of muscle cells show clearly along the left side; the dorsalmost row of the right side also shows and between these two is a clear line, the nerve tube (or cord); anteriorly this cord leads to the wedge-shaped light area at the anterior border of the larva, which is the sense vesicle (brain) and which is still open to the exterior. The test cells somewhat obscure the posterior portion of the tail.

PHOTO 24. Tadpole a little older than the preceding viewed from the right side. The tail is much elongated and the muscle cells, each faintly marked by a clear nucleus and dark outline, are also elongated as compared with preceding photos. Two rows of muscle cells are distinctly visible, the third also comes into view at the hinder end of the tail. There are seven or eight cells in each row as indicated by the number of nuclei. The gastral endoderm shows as a dark area in the trunk. The neural cord and tube is the clear area on the dorsal side of the trunk, its anterior limit being marked by a flattened contour line where the neural folds are just closing.



CONKLIN. ORGAN-FORMING SUBSTANCES.

RESEARCH SEMINAR OF THE MARINE BIOLOGICAL LABORATORY.

1. Zoölogy, Season of 1904.

July 5. **Work on the Life Histories of some Cestode Parasites of Fishes.** By Winterton C. Curtis.

July 8. **Conditions that Determine the Relative Position of Chromosomes in Mitosis.** By Ralph S. Lillie.

July 12. **The Physiology of the Invertebrate Heart.** By A. J. Carlson.

The following points were demonstrated by drawings and graphic records:

1. With the exception of *Mytilus*, in which the results are not conclusive, the auricles and the ventricle of the lamellibranchs (*Mya*, *Tapes*, *Venus*, *Hennites*, *Pecten*, *Cardium*) are supplied with inhibitory nerves from the visceral ganglion or ganglia. These fibers reach the heart in the renal nerves and enter at the base of the auricles. No nerves enter the heart along the aortæ or the rectum.

The heart of the prosobranchs (*Haliotis*, *Lucapina*, *Natica*, *Sycotypus*) and the tectibranchs (*Aplysia*, *Bulla*, *Pleurobranchæa*) is supplied with accelerator nerves from the visceral ganglion or ganglia. These nerves enter the ventricle at the aortic end. Some fibres may also reach the ventricle through the auricular walls. The auricular nerves enter the auricle at its base.

The heart of the nudibranchs is supplied with regulative nerves from the brain or supraœsophageal ganglion. In *Montereina* these nerves appear to be of the accelerator type only, while in *Triopha* both inhibitory and accelerator nerves are present.

The auricle of the slugs (*Limax*, *Ariolimax*) and the snail (*Helix*) is supplied with both inhibitory and accelerator nerves from the subœsophageal or pleural ganglion. These nerves enter the auricular musculature at the base of the auricle. The innervation of the ventricle of these pulmonates appears to be less uniform. The ventricle of *Helix* is supplied with both in-

hibitory and accelerator fibres from the same nerve as the auricle. These fibers enter the ventricle at the aortic end, some fibers reach the ventricle also through the walls of the auricle. The ventricle of *Limax* appears to be supplied with inhibitory nerves only, which enter it through the auricular walls. The ventricle of *Ariolimax* is supplied with accelerator, and probably also inhibitory fibres from the same source as the auricle. The fibres enter the ventricle at the aortic end. In *Helix* the influence of the inhibitory fibres on the ventricle, in *Ariolimax* that of the accelerator, is the greatest.

The systemic and the gill hearts of the cephalopods (*Octopus*, *Loligo*, *Ommastrephes*) are supplied with inhibitory fibres from the visceral nerves, and there is some evidence that accelerator fibres reach these hearts from the same source. In the squid the fibres to the systemic ventricle and to the auricles leave the visceral nerves at different levels. The rhythmically contracting parts of the renal veins are probably supplied with inhibitory fibres from the visceral nerves. The visceral nerves also send fibres to the pulsating vena cava. Their function was not made out.

The heart of crustaceans is supplied with inhibitory and accelerator fibres from the thoracic ganglion. In *Palinurus* the inhibitory and the accelerator fibres reach the heart along two separate pairs of nerves.

The heart of *Limulus* is supplied with inhibitory nerves from the posterior end of the brain or periœsophageal ganglion, and with accelerator nerves from the abdominal ganglia. These nerves enter the nerve-cord on the dorsal side of the heart. There is some evidence that the heart of spiders and insects is supplied with inhibitory nerves from the brain or the thoracic ganglia.

2. The action of the inhibitory nerves on the invertebrate heart is the same as the action of the vagus fibers on the vertebrate heart. The accelerator nerves in the molluscs produce contractions in the quiescent heart. Single induced shocks applied to the cardiac nerves are usually without influence on the heart unless of considerable intensity.

3. The latent period of the heart-muscle is less than 0.03″ in

the lamellibranchs and the gasteropods; in the cephalopods it does not exceed 0.02″; in the crustaceans it does not exceed 0.01″. The latent period of the heart-muscle is not greater than that of the body-muscle of the same animal.

4. The rate of propagation of the contraction in the tunicate heart (*Ciona*) varies from 200 to 350 mm. per sec.

5. The molluscan, the arthropod, and the tunicate heart exhibits a condition of reduced excitability at the beginning of systole, but no refractory period in the sense of inexcitability.

6. The molluscan, the arthropod, and the tunicate heart, that is greatly fatigued or injured, and in poor condition so that it does not beat spontaneously, responds to stimuli of increasing intensity with contractions of increasing amplitude; but the pulsating heart or the quiescent heart whose excitability is not greatly reduced tends to respond with contractions of uniform amplitude to stimuli of increasing intensity within a wide range, but increasing the strength of the stimulus above this range is followed by supermaximal contractions. The "all-or-none" law does not apply.

7. In the molluscan and the crustacean heart a strong induced shock sent through the heart at the beginning of systole diminishes the strength of that beat.

8. The molluscan, the arthropod, and the tunicate heart is inhibited in diastole by the interrupted current of a certain intensity applied directly to the heart. This inhibition is obtained in hearts apparently not provided with inhibitory nerves, and in hearts provided with inhibitory nerves after these have been paralyzed by the action of drugs. It is therefore an action of the induced current directly on the rhythmical tissue.

9. The molluscan, the arthropod, and the tunicate heart can be tetanized.

July 15. **Feeding Experiments for Determining the Life History of an Oyster Parasite.** By D. H. TENNENT.

The experiments described were conducted for the purpose of determining the adult form and the life history of the trematode, of which *Bucephalus haimeanus* Lacaze Duthiers (*Bucephalus cucullus* McCrady), abundant in oysters of various regions, is the cercaria.

An account of the varying degrees of infection, from that of the presence, in the visceral mass, of a simple unbranched sporocyst, to the almost complete replacement of the gonads and liver by the long germ tubes, or branches of the sporocyst, was first given.

The first experiments described were conducted for the purpose of determining whether infection might spread directly from one oyster to another. Five experiments covering about one year, were mentioned.

In the second experiments conducted, an attempt was made to obtain the adult form by feeding infected oysters to fish which were kept in aquaria. These experiments seemed to show conclusively that the host of the adult form of *Bucephalus* was not necessarily an oyster-eating fish.

While these experiments were being carried on, a search for *Gasterostomum*, to which *Bucephalus*, by reason of its structure, would seem to be intimately related, was being made. The animals and plants found in the regions in which infected oysters occur, were thoroughly examined.

Gasterostomum was ultimately found in abundance in the garfish, *Tylosurus marinus*.

After determining the food of the gar, collections of the various fish and crustacea included in this list were examined.

In the viscera of the silverside, *Menidia notata*, specimens of which had been found in the stomach of the gar, encysted forms, little different in appearance from the cercariæ present in the oyster, were obtained.

The final set of experiments consisted in feeding viscera of the silverside to four species of fish, previous examinations having shown that in these species *Gasterostomum* does not occur.

These experiments showed that the encysted forms from the silverside were able to resist the action of the digestive juices of the fish to which they were fed and that they attached themselves to the wall of the intestine of their new host and continued in their development.

The evidence afforded by these experiments and observations showed that during its life history *Gasterostomum*, parasitic in the digestive tract of *Tylosurus marinus*, passes its cercaria stage

(*Bucephalus haimeanus*) in the oyster; that the cercariæ becoming mature leave the oyster and swim about in the water; that they are from thence obtained as food by the silverside and other small fish; that in these fish they encyst, finally to be released, and become sexually mature when their hosts are eaten by *Tylosurus*.

The extent of infection, the character of the regions in which infected oysters are found, the effect of the parasite upon the oyster, the origin of the germ cells from which the cercariæ arise and the significance of the differences in appearance and rate of growth in the experiment fish were also mentioned.

The experiments described were conducted at the Beaufort, N. C., Fisheries Laboratory.

July 19. **Relation of Ants to Temperature and Submergence.** By ADELE M. FIELD.

An artificial nest was made, having as its basis a half-cylinder of copper, with ends projecting beyond the nest. One end of the copper was heated by an alcohol flame, while the other end was surrounded by ice. Different parts of the nest presented diverse temperatures, ranging from 10° C. or 14° F. to 60° C. or 140° F. Ants of various species were introduced into this nest, and it was found that the temperature preferred by them was from 24° C. or 76° F. to 27° C. or 82° F. Below 15° C. or 60° F. the ants become sluggish, and increase of temperature was accompained by increase of activity. Above 30° C. or 86° F. they manifested discomfort or distress. Exposure to heat so great as 50° C. or 122° F. for a period of fifteen seconds for the smaller ants, or of two minutes for the larger ants, invariably killed them, probably by coagulation of their protoplasm. The effect was the same whether the heat was applied through a wet or dry medium.

Ants of the same species as those killed by heat, survived submergence in cool water for seventy-two hours. The futility of ploughing up ant-nests with the expectation that the spring rains would destroy the ants was alluded to, and the application of heat was suggested as a better means of destroying these pests on farms.

July 22. **Habits and Life-History of Parasitic Copepods.** By CHARLES B. WILSON.

A statement was first made of some of the problems confronting the student of this group, for only one of which has there been any attempt at solution. This is the one which logically comes first, the determination and description of species. But it is the least important and should be quickly followed by a careful study of habits, including relative abundance and the influence of the parasite upon its host. The life-history must also be worked out before adequate methods can be devised for exterminating the parasites or checking their ravages. Then come a series of ecological problems for which these copepods afford exceptionally fine material, color protection, mimicry, adaptation to environment, degeneration phenomena, and the like.

Material was shown for several of these problems, with a series of drawings for the life-history of the Caligidæ, the largest family in the group. The history of three members of this family, *Caligus rapax*, *Caligus bonito* and *Alebion glabrum*, was presented in full, that of others only partially.

The eggs hatch into a typical nauplius which swims about freely at the surface. After the second or third moult it becomes a metanauplius whose second antennæ cease to function as locomotor and become prehensile. At this stage it seeks its host and fastens to it.

In *Caligus*, *Lepeophtheirus* and allied genera an attachment filament is developed at the next moult from a median frontal gland which holds the larva securely to its host until it has matured. In *Alebion* and allied genera the second antennæ continue to serve as organs of attachment up to maturity. In the metanauplius there are but two pairs of thoracic appendages; a pair is added at each successive moult up to the normal number.

These parasites do not usually injure their host, but may become sufficiently numerous to cause serious damage, especially if the host has been weakened or injured.

They feed upon the blood of their host which they obtain by piercing the skin in the vicinity of blood vessels and extracting the blood by means of a suctorial mouth tube.

They often become a serious nuisance to the artificial breeder

of fish in ponds or aquaria. The only remedy in the case of the adults is to remove them carefully from the fish, but further trouble can be avoided by a plentiful introduction of small surface fish, sticklebacks, minnows, etc., which will eat up the larvæ of the parasitic copepods.

July 26. **The Nature of the Heart-Rhythm.** By A. J. CARLSON.

The following points were demonstrated on the heart of *Limulus*.

1. The heart-beat is neurogenic, not myogenic. The heart-muscle is not automatic under the normal conditions of life. Extirpation of the ganglion or nerve-cord on the dorsal side of the heart abolishes the rhythm at once and permanently, the muscle contracting only on artificial stimulation.

2. Coördination or conduction in the heart takes place in the nervous and not in the muscular tissue. The entire heart, save the median nerve-cord and the lateral nerves, may be cut transversely. This does not affect conduction or coördination. Severing the nerve-cord and the lateral nerves, leaving the heart-muscle intact, abolishes coördination of the ends of the heart at either side of the lesion, the contraction not passing the level of the lesion in either direction.

3. The inhibitory nerve-fibers act on the ganglion cells in the heart and not on the heart-muscle. Cardiac inhibition falls within the category of inhibition of automatic or reflex neural processes.

August 2. **Toxic and Antitoxic Action of Salts.** By ALBERT P. MATTHEWS.

August 6. **Causes of Blue and Green in Feathers.** By R. M. STRONG.

There are no blue pigments known in feathers, excepting one possible case cited by Häcker, and green feathers rarely owe their colors to green pigments. The blues are so-called structural colors confined to the barbs, usually.

Walter's hypothesis that these phenomena are *surface* colors produced by underlying melanin pigments is untenable because the pigments when isolated are not blue but dark brown.

There are serious objections to the turbid medium hypothesis of Häcker and others. Experimental studies indicate that most

of the blue is produced, not in the horn substance of the feather, but at the dorsal inner surfaces of medullary cells where the incident light passes from the horn substance into the air filling the cavities of the medullary cells. The blue color is probably due to the stronger refraction of the blue end of the spectrum with consequent greater reflection of the blue.

The greens are usually produced by feathers which do not differ essentially from blue feathers except in having a yellow pigment in the cortex. The yellow pigment absorbs the incident blue, but transmits the green rays. The latter are reflected more strongly than the rays on the red side of the green in the spectrum, and they consequently give the feather its green color.

Many variations occur in the shape of blue and green barbs, and the latter very commonly have a high elevation of the dorsal cortex which contains a diffuse yellow pigment. The barbules are usually reduced or absent.

In some species, we find the green changing to dark dull yellow when the angle of incidence becomes very large. This phenomenon does not belong to the category of the common metallic or iridescent colors. It depends on purely mechanical conditions. The green disappears when the angle of incidence becomes so great that only the dorsal, yellow cortical elevations of the barbs are in view.

August 9. **Rheotropism in Fishes.** By E. P. Lyon.

It seemed impossible to the author that pressure could be the method of stimulation which brought about orientation of fishes in a current of water, according to the usual theories on the subject. The effect of the current would seem to be simply to carry organisms having approximately the same specific gravity as water down stream. No pressure would result unless the animal became oriented and swam against the current. It therefore seemed that points of reference on the bottom or banks must be as important in stimulating the fish as the current itself. Testing this hypothesis it was found that the fish responded to any movement of the bottom of the dish in which they were kept, turning in the same direction as the moving objects. By a series of experiments it was definitely made out that by far the largest ele-

ment in securing orientation of the fishes experimented with is an optical reflex of such a kind that the animal tends to retain the same visual field. It is found however that blind fishes or fishes in the dark are able to orient themselves. Investigation of this phenomenon showed that it is primarily due to objects on the bottom. Blind fishes do not orient themselves in a uniform current unless they touch objects which are stationary. In more violent streams of water where there are considerable differences of velocity between closely proximated parts of the stream, orientation may occur. Here apparently the same explanation holds, *i. e.*, that the relatively stationary water constitutes the reference points by which the animal establishes its direction in the stream.

It is believed that pressure in a gross, mechanical way cannot explain rheotropism, but rather that it is always a response to the relative motion between the fish being carried passively down by the moving water, and more stationary parts of the environment.

August 12. **Osmotic Pressure of Sea Water and Marine Animals.** By W. E. GARREY.

1. The osmotic pressure of the sea water was determined by testing the depression of the freezing point (Δ) with a Beckmann apparatus.

(*a*) Buzzards Bay water, $\Delta = -1.83°$ and $-1.82°$.

(*b*) Eel Pond water, $\Delta = -1.77°$.

(*c*) Salt water tap of Marine Biological Laboratory showed considerable variations; $\Delta = -1.86°$ in the early part of the season and as low as $-1.78°$ after heavy rains. Most of the readings however varied between $-1.84°$ and $-1.82°$.

(*d*) Basin of Fish Commission $-1.84°$ and $-1.81°$.

These waters thus show a freezing point slightly less than that of molecular cane sugar ($\Delta = -1.85°$) and the osmotic pressure is about 22 atmospheres calculated at 0° C.

2. The body fluids of marine invertebrates have an osmotic pressure very close to that of the sea water from which they are taken.

3. By immersion in diluted or concentrated sea water marine invertebrates take up or lose water respectively and assume an osmotic pressure approximating that of the external medium.

4. Selachians have an osmotic pressure equal to that of the sea water and change with the external medium, although not so readily or completely as invertebrates. They die if the change in osmotic pressure is great.

5. Marine teleosts have an osmotic pressure only about half that of the sea water; $\Delta = -0.80°$ and $-0.96°$. Transferring common eels from salt into fresh water did not lower the osmotic pressure of their blood. *Fundulus Heteroclitus* could also be placed in fresh water or doubly concentrated sea water and live for weeks. If, however, the body was partly scaled, or part of the skin removed, the fish die in normal sea water and in fresh water, but can be kept indefinitely in sea water which has been diluted with an equal volume of distilled water and which therefore has an osmotic pressure approximating that of their blood. These experiments point to the normal impermeability of the teleost integument.

August 12. **Coagulation of the Blood.** By LEO LOEB.

In former investigations I made it very probable that the so-called first coagulation of the blood of certain arthropods and the coagulation of the blood of *Limulus* consists in an agglutination of blood cells, that in the second coagulation of arthropods, on the other hand, fibrin is formed from fibrinogen under the influence of substances present in the blood cells and in the tissues. The latter, which I called tissue coagulins, are, within certain limits, specific, the former being not at all or much less specific. Similar conditions are present in vertebrate blood.

We have therefore to distinguish between two kinds of substances which may, under ordinary circumstances, affect the coagulation of the blood, namely, substances present in the serum and those present in the tissues (tissue coagulins).

Quite recently several investigators (Morawitz, Fuld u. Spiro) have advanced the theory that the tissue coagulins act only indirectly by transforming (to use the terms of Morawitz) thrombogen into prothrombin, which, by the aid of calcium, becomes the active ferment. This is a somewhat modified form of Alexander Schmidt's theory of the coagulation of the blood. Certain facts, however, made it appear to me more probable that

the tissue coagulins attack the fibrinogen directly and transform it into fibrin. That this actually does take place I believe to have been able to show in the invertebrate blood (lobster), insofar as tissue coagulins still cause the coagulation of the blood plasma, after the substances present in the blood which accelerate the coagulation of the blood have been previously destroyed by heat.

It seems permissible to apply this result to vertebrate blood, the similarity between the factors causing the coagulation of vertebrate and invertebrate blood being very great.

In connection with the foregoing experiments a comparative study of the conditions under which these two substances act was made (influence of dilution, of calcium and other salts, power of resistance, preparation of active extracts). Their action on artificially prepared fibrinogen was also compared.

New facts were found which support the view formerly expressed that the coagulation of other arthropods represents an agglutination of the blood cells. In accordance with this view, I could prepare fibrinogen from lobster blood, in which a second coagulation takes place. On the other side, no fibrinogen could be prepared from *Limulus* blood, which has no second coagulation.

2. BOTANICAL LECTURES AND SEMINARS IN 1903.

July 8, Dr. George T. Moore: "The Polution of Public Water Supply by Algæ."

July 10, Dr. Bradley M. Davis: "The Relationships of the Sexual Organs of Plants."

July 20, Dr. Henry Kraemer: "The Unorganized Contents of the Plant Cell."

July 21, Dr. Henry Kraemer: "The Structure of the Cell Wall."

July 27, Mr. Herbert J. Webber: "History and General Factors of Plant Breeding."

July 28, Mr. Herbert J. Webber: "The Principles of Selection and Isolation in Improving Breeds."

July 29, Mr. Herbert J. Webber: "The Mutation Theory of De Vries."

July 30, Mr. Herbert J. Webber: "The General Laws of Hybrids with a Discussion of Mendel's Principles."

July 31. Mr. Herbert J. Webber: "The Utilization of Hybrids in Practical Plant Breeding and the Selection of Vegetative Parts in Breeding."

August 5, Dr. Bradley M. Davis: "Cytoplasmic Structures of the Plant Cell."

August 6, Dr. Rodney H. True: "Theory of the Nature of Solutions. Dissociation Hypothesis and Objections to it."

August 7, Dr. Rodney H. True: "Toxic Action of Solutions on Plants, caused by Solutions Containing One Solute."

August 10, Dr. Rodney H. True: "Toxic Action of Solutions on Plants Caused by Solutions containing more than one Solute."

August 11, Dr. Rodney H. True: "Influence of Solutions on Plant Functions and Structure."

August 11, Mr. Christopher S. Oglevee: "The Influence of Insoluble Substances on the Action of Poisons in Solutions."

August 12, Dr. Rodney H. True: "Artificial Sea Water."

July 8. **The Pollution of Public Water Supplies by Algæ.** By GEORGE T. MOORE.

The importance of a scientific investigation of this most common cause of the bad odors and tastes in drinking water was pointed out and numerous examples were given of the serious inconvenience and great financial loss due to the presence of algæ in reservoirs. It is necessary to recognize that the means of finding a remedy for the trouble must be biological rather than chemical or mechanical, and it is only after we are familiar with the life history of the organisms producing the odor and taste that we can hope to find a remedy.

Certain experiments then under way, being carried on by the Department of Agriculture, were described, and it was believed that the result of these trials would prove the discovery of a cheap and practical remedy which could be used on the largest scale and yet not cause trouble to the consumer.

July 10. **The Relationships of the Sexual Organs of Plants.** By BRADLEY MOORE DAVIS.

The types of sexual organs found in plants, their structure, origin and relationships were considered. These types fall into

three classes: (1) the unicellular sexual organs (gametocysts) (2) the multicellular sexual organs (gametangia) and (3) the peculiar multinucleate gametocysts which become sexual cells (cœnogametes). The first class (gametocysts) is found throughout the Thallophytes, and the evolutionary principles governing their differentiation into spermatocysts and oöcysts are well understood. The second class (gametangia) is characteristic of the bryophytes and pteridophytes and contains the well-known antheridium and archegonium of these groups. The possible origin of these structures from the plurilocular sporangium, according to the writer's recently published hypothesis, was discussed. The third class (cœnogametes) comprises the remarkable cœnocytic sexual cells found among the Mucorales, Saprolegniales and Peronosporales. Great interest attaches to their behavior and phylogenetic relations on account of the peculiarities of their structure, which are not found in any other group of organisms. The author's explanation of these conditions was presented in the light of his recent investigations on oögenesis in *Vaucheria*.

July 20. **The Unorganized Contents of the Plant Cell.** By HENRY KRAEMER.

The micro-chemical and micro-physical properties of the following unorganized cell contents were considered: (1) Product of constructive metabolism; the crystalloidal carbohydrate, starch. (2) Products of reconstructive or destructive metabolism; *A*, organic substances — (*a*) occurring in the protoplasm and cell-sap, as oils, resins, ferments and proteine crystalloids; (*b*) occurring in the cell-sap, as crystalline carbohydrates (dextrose, maltose, sucrose, etc.) and the crystalloidal carbohydrate inulin; (*c*) occurring in cell-sap or cell-wall, colloidal carbohydrates (gums, mucilages and pectins), tannin, alkaloids, glucosides and calcium oxalate; *B*, inorganic substances — (*a*) calcium carbonate in cystoliths or in the cell-wall; (*b*) silica in irregular masses or in the cell-wall.

July 21. **The Structure of the Cell-wall.** By HENRY KRAEMER.

The various methods for differentiating the different lamellæ of the cell-wall were first considered. The similarity in the

structure of the starch grain and that of the cell-wall was demonstrated by micro-physical means, the use of iodine solutions and aniline stains. The various types of cell-walls and modified cell-walls were described as follows: (1) Cellulose walls; (2) ligno-cellulose walls; (3) adipo-cellulose walls; (4) mucilage-cellulose walls; (5) pecto-cellulose walls; (6) reserve-cellulose walls. In connection with the study of the reserve-cellulose walls the work of the author on the continuity of protoplasm was considered, and the similarity of the structure of the walls of this class to the structure of the wheat starch grain was pointed out.

July 27. **History and General Factors of Plant Breeding.** By HERBERT J. WEBBER.

This lecture included a discussion of the history of breeding from the time of Fairchild who made the first plant hybrid in 1719 to the present time. The principles of breeding advocated by Knight and Van Mons were compared with later methods and the gradual improvement traced.

Illustrations were given of physiological variations, individual or congenital variations and mutations or saltatory variations, showing how the two latter forms of variations have been utilized by practical breeders, while physiological variations have proven valueless because not hereditary.

The factors of breeding, including the general laws of heredity, transmitting power, unity of individual isolation and selection, were considered briefly.

The speaker emphasized the importance of breeding for a special purpose and with a definite idea in view. The breeder should be familiar with all varieties, races, and species of the plant which he proposes to improve, and select as the parent or parents for his new sort, the existing variety or varieties which exhibit in the greatest perfection the ideal characters which he desires to combine.

In the amelioration of a wild species it has been claimed since the time of Knight that it is first necessary to "break the type" as it is expressed, to get the plant into a condition of variation. It is claimed that a wild plant is for some time very stable and but slightly variable under conditions of cultivation, and that by continuous high cultivation this stability can be broken and the

plant changed into a condition of variability, a condition which would appear comparable to the mutation period predicated by De Vries. The evidence in favor of the theory of breaking the type is slight being based mainly on the experiments of Knight and H. Vilmorin.

July 28. **The Principles of Selection and Isolation in Improving Varieties.** By HERBERT J. WEBBER.

This lecture described in detail the methods of selection used in originating new races and strains of wheat, corn, cotton, sugar, beets, and other agricultural crops. The nursery method of planting introduced by Hallett and used extensively in this country by Professor Hays and others was compared with the field method of selection introduced by Rimpau, and illustrations were given of the use of each method in the production of new forms. The nursery method gives to each plant all the space it requires and allows the plant to show what it will do under the most favorable conditions. The field method provides for the growing of the plants under the conditions of field culture where different individuals compete with each other in a struggle for existence, the same as occurs in the ordinary conditions of culture under which the plant is grown.

The importance of considering the individual as the unit of selection under ordinary conditions was emphasized but it was pointed out that in some cases the selection of a fraction of an individual will give quicker results. In corn for instance, the kernels on an ear may show several different colors and it was demonstrated that a uniform color can be secured more quickly by separating out these kernels which show the desired color. In hybrids of smooth and fuzzy-seeded cottons on the same plant, some bolls may have nearly smooth seeds, while others have fuzzy seeds in various degrees. It has been found that the seeds in a single boll run very uniform either smooth or fuzzy, and the evidence obtained on this point indicates that a larger percentage of individuals producing smooth seed can be obtained by selecting seed only from bolls producing smooth seed. These points were emphasized to show that breeders must be prepared to take advantage of every important point that may appear.

July 29. **The Mutation Theory of De Vries.** By HERBERT J. WEBBER.

The speaker called attention to the fact that very many of the races and varieties of our cultivated plants have originated as sudden variations. Individual variations were discussed in comparison with mutations and it was claimed that no strict line of demarkation can be drawn between the two. The work of selections deals mainly with slight individual variations, some of which as shown by experiment have strong transmitting power and reproduce themselves true to type in large degree. No means exists of distinguishing between these and small mutations which are mainly transmitted true through the seed. The difference is one of degree only, it was claimed, and cannot be detected with certainity.

The influence of natural selection in the origin of natural species is not eliminated by accepting the idea that variations that form species are produced as mutations. Mutations of all kinds are doubtless formed, desirable and undesirable, fit and unfit, and only those maintain themselves and form permanent species that are fully in harmony with the environment and thus survive. Undesirable mutations are weeded out by natural selection. Even granting the occurrence of a mutation strikingly different from the parent type and thoroughly fitted to the environment, something more is necessary other than natural selection to insure its forming a new species. It is of primary importance that the mutation or variation have strong transmitting power, giving progeny like the parent mutation. Aside from this, some form of isolation is necessary to secure the formation of the new type as the few plants showing the variation would be swamped by panmixia. This swamping the speaker pointed out could be overcome or avoided in at least three ways; namely, (1) geographical isolation, (2) tendency to self-fertility, or prepotency of pollen, (3) a tendency to prepotency or preponderance of type. The first of these, the influence of geographical isolation, has been emphasized by Gulich and Romanes, and its general application is familiar to all through the classical illustration of snails in different valleys in the Sandwich Islands given by Gulich. The influence of the other two factors the speaker has never seen

emphasized although he is inclined to believe that they are very important factors in evolution. The second factor which is a form of isolation, namely tendency to self-fertility or prepotency of pollen, would be present whenever the flowers of the plant are modified to insure self-fertilization, as in the case of the pea, bean, and many other legumes, in wheat, barley, and some other plants, and in cases which may occasionally occur where the mutation gives rise to a plant which has a strong tendency towards self-fertility, the progeny of cross fertilization with pollen of another individual being fewer in number and lacking in vigor. Such a mutation was observed and experimented with by Darwin and graphically described under the caption "The Hero Morninglory."[1] The races of Cupid sweet peas, and various wheat races are illustrations of cultivated species, we may call them, that have originated in this way. Given a mutation suited to the environment, having strong transmitting power, and a tendency to self-fertility, or some device insuring self-fertility and we have the creation of a new species.

The third means by which the swamping effect of panmixia may be overcome is where a tendency to prepotency or preponderance of type is formed. In such instances the mutation, giving rise to a type which while not necessarily preferring self-fertilization is strongly prepotent and dominant in its action and transmits its characters to all its progeny which bred *inter se*, would probably strengthen the type and lead to its gaining a permanent foothold. Such illustrations, like the Ancon or Otter sheep are not uncommon among domestic animals and plants.

July 30. **The General Laws of Hybrids with a Discussion of Mendel's Principles.** By HERBERT J. WEBBER.

The claim advanced by Mendel and some of his followers that of a certain allelomorph or character pair, one character is dominant and masks the other character in first generation hybrids was claimed by the speaker to have but limited application. Many cases of blends of two characters were cited. It was also pointed out that the statement that first generation hybrids are of the same type is erroneous. The case of hybrids of orange

[1] Darwin, Cross and Self-fertilization in the Vegetable Kingdom," p 47.

(*C. aurantium*), trifoliate orange (*C. trifoliata*), where each seedling differs from every other seedling, was cited.

It was shown that the conception of purity of the germ cell which is the primary feature of Mendel's laws must certainly be modified as the fact of reversions to ancestral characters in types that have bred true for generations show that the "Analage" of the character must have remained in the germ cell through all these generations without exerting or showing itself. Cases of reversion in cotton and other plants were cited as illustrations.

The principle of the segregation of characters in the formation of the germ cells was also denied from the fact that many hybrids have been bred into fixed races coming true to seed that exhibit a heterozygote character intermediate between the two parental characters. Such races are the white cap dent corn, a hybrid of a yellow and a white dent which is yellow on the sides and white on the apex of the kernel; the Griffin, Allen hybrid, and Doughty cottons which are hybrids of sea island and upland cotton that have fibers intermediate between the two parents in length and fineness.

Mendel's researches were considered by the speaker to be of the utmost importance from the theoretical scientific standpoint, but it was claimed that they will have little or no effect in changing the methods of practical breeding.

July 31. **The Utilization of Hybrids in Practical Plant Breeding; and the Selection of Vegetative Parts in Breeding.** By HERBERT J. WEBBER.

While pure selection gives the quickest and surest results in breeding, it is to hybridization that we must give attention when we desire to produce striking new sorts or combinations of the good qualities of two or more existing sorts with the elimination of the undesirable features. It is very necessary to carefully select the races or species to be combined, and also just as necessary to select the best individuals of these races or species that are to be used as the parents.

The second generation is the variable hybrid generation from which our selection of desirable combinations must be made. This is in accordance with Mendel's conclusions but was known

and used in practice long before the rediscovery of Mendel's works.

The fixation of select hybrids into stable races is best accomplished by isolating the progeny of the select plant and breeding together those individuals of the progeny which are nearest alike. Probably fixity or uniformity could be gained quicker by in and in breeding, but in plants that are normally cross fertilized the loss of vigor and fertility from the inbreeding would probably in most cases render the use of this method impractical.

Attention was directed to the important improvements that can be secured by the selection of vegetative parts. By the selection of vigorous growing cuttings of the carnation, violet, rose, etc., the size and number of the flowers produced can be greatly increased. Many bud sports or bud mutations have been used as new varieties and are valuable acquisitions.

Plant breeding, it was pointed out, is not necessarily a difficult task. The speaker urged the great importance of the work and the necessity of more extensive scientific investigations and practical experiments.

August 5. **Cytoplasmic Structures of the Plant Cell.** By BRADLEY MOORE DAVIS.

The writer described in this seminar the principal structures and activities of the cytoplasm in different parts of the plant cell and at different periods of ontogeny. There are three principal regions of the cytoplasm: (1) The plasma membranes (kinoplasmic in character), which surround the protoplast, the vacuoles and the nucleus, (2) trophoplasm, and (3) the kinoplasm intimately concerned with mitotic phenomena and which finds morphological expression in asters, centrospheres, centrosomes and filarplasm. The structure of trophoplasm was described together with the peculiar physodes, nematoplasts and cœnocentra. But the main discussion concerned the behavior of kinoplasm during mitosis and the segmentation of the protoplasm at cell-division. Many of the kinoplasmic structures characteristic of these events may be closely related to one another, in spite of their diverse morphology, when studied ontogenetically, and the author discussed some of these problems with especial reference to his studies on the Hepaticæ and Thallophytes.

August 6. **Theory of the Nature of Solutions. Dissociation Hypothesis and Objections to It.** By RODNEY H. TRUE.

August 7. **Toxic Action of Solutions on Plants Caused by Solutions Containing One Solute.** By RODNEY H. TRUE.

August 10. **Toxic Action of Solutions on Plants Caused by Solutions Containing More than One Solute.** By RODNEY H. TRUE.

August 11. **Influence of Solutions on Plant Functions and Structure.** By RODNEY H. TRUE.

These seminars presented a discussion of the theory of electrolytic association and its bearing on plant physiology. The theory was outlined and some of the more recent objections to it were stated, together with some of the more important grounds on which such objections are based. Attention was called to the bearing of these objections, with reference to physiological research along these lines, and caution was advised in construing results in terms of the theory. It was pointed out that, as long as a considerable mass of evidence lies against this hypothesis, physiologists should not commit themselves too thoroughly to the theory and its conclusions. The toxic action of various classes of compounds was then discussed in the light of the dissociation hypothesis and the relation between physiological action and molecular structure was pointed out. Some relations obtaining between the structure and functions of plants and the action of molecules and ions was discussed.

August 11. **The Influence of Insoluble Substances on the Action of Poisons in Solution.** By CHRISTOPHER S. OGLEVEE.

Seedlings of *Lupinus albus* were grown in beakers containing the various poisons. The concentration which would kill the radicle was determined. It was found that finely divided particles of an insoluble substance placed in the beaker containing the poisonous solution not only allowed the radicle to grow, but often accelerated the growth above the normal, and produced effects similar to those of a more dilute solution. The insoluble substances used, sand, pounded Bohemian glass, shredded filter paper, potato starch, and paraffine, were cleaned and washed as

thoroughly as possible. Some of the poisons used were copper sulphate, silver nitrate, mercuric chloride, citric acid, resorcin, phenol, thymol, etc. The effects could not be due to the action of the insoluble particles on the ions of the poisons which do not dissociate. The variety of insoluble substances used would indicate that the effects were not due to the slight solubility which some of the particles possess. Since no other suggestion presents itself, perhaps the theory of "adsorption" offers the best explanation to the problem.

August 12. **Artificial Sea Water.** By RODNEY H. TRUE.

The experiments now in progress at the United States Fish Commission in connection with the attempts to make artificial sea water capable of sustaining marine life were discussed. Two sorts of solutions were compounded, one a synthetic, prepared by adding to distilled water the required salts in quantities indicated by analysis. The second solution was obtained by dissolving in distilled water a complete sea salt obtained by evaporating sea water to dryness over steam. The importance of the presence of the full amount of calcium was pointed out and the utility of charging the solutions with carbon dioxide gas was noted. The report of the results thus far obtained with the marine plants and animals indicated that the synthetic solutions are less satisfactory than those obtained from evaporated sea salt. The hope was expressed that the latter might be to a considerable degree satisfactory for the demands in this direction, but the fact that experiments thus far have covered only two summer sessions was cited as ground for a cautious interpretation of these results.

3. BOTANICAL LECTURES AND SEMINARS IN 1904.

July 12, Dr. George T. Moore: "Botanical Work at Several Botanical Laboratories in Holland, Scandinavia and Russia."

July 13, Dr. George T. Moore: "The Physiological Methods of Purifying Water Supplies."

July 15, Mr. Mintin A. Chrysler: "Regeneration in Plants as Illustrated by Zamia Floridana."

July 19, Miss Etoile B. Simons: "Morphological Studies on Sargassum Filipendula."

July 22, Dr. James J. Wolfe: "Cytological Studies on Nemalion."

July 27, Dr. Henry Kraemer: "The Origin and Nature of Color in Plants."

August 1, Dr. G. F. Blakeslee: "Sexual Reproduction of the Mucorineæ."

August 10, Dr. Bradley M. Davis: "The Algal Flora of the Bay of Naples."

July 12. **Botanical Work in Several Botanical Laboratories of Holland, Scandinavia and Russia.** By DR. GEORGE T. MOORE.

A popular account of a recent visit made to some of the principle botanical laboratories in the countries mentioned. A description of the facilities for work at Delft, Amsterdam, Copenhagen, Christiania, Bergen and Stockholm, with a brief reference to the principle botanists in these cities was given, as well as a more detailed account of the Institute for Experimental Medicine at St. Petersburgh and its illustrious director, Winogradsky.

July 13. **The Physiological Method of Purifying Water Reservoirs.** By DR. GEORGE T. MOORE.

After a brief reference to the very widespread difficulty in water supplies due to algæ, and the failure to devise any means of removing or preventing the bad odors and tastes, an account of the toxic effect of copper sulfate upon algæ was given. It has been known for a long time that many of the heavy metals were extremely poisonous to some plants, and experiments showed that copper sulfate could be used at a dilution so high as to be absolutely harmless to man and yet sufficient to destroy or prevent the growth of algal pests. Practical application of the method to reservoirs containing millions of gallons has shown that the necessary requirements of efficiency, cheapness and harmlessness to man are all complied with and it is believed that a thoroughly reliable means has been devised for preventing the growth of algæ in water supplies.

While the application of the method to the destruction of typhoid and cholera is not universal as with the algæ, it is believed that under certain conditions the use of copper sulphate

offers the only known means of thoroughly and quickly sterilizing a reservoir.

Extensive experiments on a large scale are now being carried on and results already obtained warrant the conclusion that the method is satisfactory in every respect.

July 15. **Regeneration in Plants as Illustrated by Zamia floridana.** By MR. MINTIN A. CHRYSLER.

Zamia floridana exhibits a remarkable capacity for producing new shoots and roots from pieces of stem. A piece no larger than a walnut can give rise to a new plant. The new shoots proceed in most cases from the vascular region of the central cylinder, but may arise from the neighborhood of the periderm, or may form a cap over the whole central cylinder. Only the last case merits the term "regeneration"; the others are merely instances of adventitious budding. Roots may spring from the cut surface just as do the shoots. The power of producing new shoots seems to be shared by all tissues which have remained meristematic, or which have resumed the power of cell division such as the phellogen of the wound cork. A case of budding from the hypocotyl has been observed. The theories of "latent buds" and "polarity" are not supported by the specimens of *Zamia* examined.

July 19. **Morphological Studies on Sargassum Filipendula.** By MISS ETOILE B. SIMONS.

The conceptacle, antheridium, oögonium, and cryptostoma were discussed in detail.

Sargassum Filipendula is unlike the accounts of authors who have studied various other members of the Fucales in the development of its conceptacle.

Bower in his article "On the Development of the Conceptacle in the Fucaceæ" describes a single superficial initial cell, which usually disintegrates. According to his account cells adjacent to this initial divide and form the conceptacle. Holtz announces a group of initial cells in *Pelvetia fastigiata* which disintegrate. Below these and other disintegrating cells are found the elements which form the conceptacle walls.

The conceptacle of *Sargassum* originates with a single superficial cell. Cell division beginning in this initial continues in the progeny until the conceptacle is formed. The entire organ, therefore, is the descendant of one cell, the true initial.

The antheridium and oögonium develop from wall cells of the conceptacle as in other members of this group. The oögonium has no pedicel, however, and usually contains but one egg.

The cryptostoma develops from a single superficial cell as does the conceptacle and is homologous with that organ.

July 22. **Cytological Studies on Nemalion.** By DR. JAMES J. WOLFE.

The chromatophore of *Nemalion* has a hollow ellipsoid form from which processes radiate to the periphery of the cell and there flatten out to form a clathrate membrane. The region surrounded by the ellipsoid portion of the chromatophore, and generally regarded as a pyrenoid, consists entirely of vacuolar material.

The sex-organs cannot be regarded as unicellular structures; since in earlier stages the trichogyne possesses a well organized nucleus, which fragments as that organ matures; the egg-cell thus becoming an intercalary cell, and the trichogyne one which has been specialized in connection with the reproductive processes.

The nucleus of the spermatium normally divides into two fertilizing elements which are discharged into the trichogyne; which events show the so-called spermatium to be an antheridium.

In the mature cystocarp the ultimate cells give rise terminally, as well as by subterminal proliferation, to a variable number of carpospores, which is further augmented by repeated proliferation within the successively formed mother-cell walls.

The entire chromatin content of the nucleus is stored in the nucleolus, and in the prophases of division passes to the nuclear wall along delicate fibrillæ. The spindle is intranuclear, and centrosomes are distinctly visible at metaphase.

The conclusion that *Nemalion* presents the essentials of an antithetic alternation of generations, and that the cystocarp is, therefore, the homologue of the sphorophyte in higher plants, is indicated by cytological evidence — since approximately sixteen chromosomes are present in the divisions of the cells of the cysto-

carp up to the period of spore formation, and approximately eight in those of the thallus, the reduction division being immediately associated with the production of carpospores.

July 27. **The Origin and Nature of Color in Plants.** By DR. HENRY KRAEMER.

Color in plants is due to definite constituents which either themselves are colored or they produce colors when acted upon by other substances. These constituents are either associated with some of the organized bodies of the plant cells, or they occur in the cell-sap. To the former belong the pigments associated with the etioplasts, chloroplasts and chromoplasts. These are distinguished from all other color substances of the plant by their solubility in ether, benzol, xylol, chloroform and similar solvents.

Besides the plasted pigments there are substances dissolved in the cell-sap. These occur in all parts of the plant and give rise to the other shades and tints than yellow and green. They are quite soluble, usually in a 50 per cent. hydro-alcoholic solution, and are insoluble in the above-mentioned solvents. They give well-marked reactions with certain groups of reagents and show many properties in common whether obtained from flowers, leaves, fruits, roots or stems.

The relationship of the chloroplastid to the production of starch has already been pointed out. The occurrence of proteid substances in chromoplastids suggests that they have the special function in the manufacturing or storing of nitrogenous food material which is subsequently utilized in the development of the ovule, germinating plant or biennial plant. The wide distribution of cell-sap colors, which share many general properties in common, suggests that these substances, like other unorganized cell contents, are but incidental to physiological activity.

August 1. **Sexual Reproduction of the Mucorineæ.** By A. F. BLAKESLEE.

According to their methods of zygospore formation the Mucorineæ may be divided into two groups. In the homothallic group zygospores are developed from branches of the same thallus. In the heterothallic group zygospores are developed from branches

which necessarily belong to thalli diverse in character. Every heterothallic species is an aggregate of two distinct strains through the interaction of which zygospore production is brought about. These sexual strains in an individual species show a more or less marked differentiation in vegetative luxuriance which may be designated by (+) and (−) signs respectively. A process of imperfect hybridization will occur between unlike strains of different species. By taking advantage of this character it has been possible to group together in two opposite series the strains of all the heterothallic species under cultivation. When thus grouped the (−) strains will be in one series while the (+) will be in the other. In the two series are represented the two sexes.

August 8. **The Algal Flora of the Gulf of Naples.** By DR. BRADLEY M. DAVIS.

This seminar considered the character and distribution of the marine algæ in the vicinity of Naples and was illustrated by a collection. Comparisons were made with the flora of the Atlantic coast of America south of Cape Cod which has some important features in common with the Mediterranean. The opportunities for botanical work in the zoölogical station at Naples were described.

THE OSMOTIC PRESSURE OF SEA WATER AND OF THE BLOOD OF MARINE ANIMALS.

Including Some Observations on the Permeability of Animal Membranes.[1]

WALTER E. GARREY.

Experimental work on the relation of salts and other substances in solution to the life processes of marine animals requires an accurate knowledge of the osmotic pressure of both the sea water and the body fluids of the animals. This knowledge should be supplemented by definite information relative to the permeability of the membranes of the animals under investigation. Thus far this field has been neglected by American investigators. It has been assumed that local conditions are similar to those existing along the European sea board. Owing to the character of the work in our Marine Laboratories it seemed advisable to make some observations which would place our knowledge of local conditions on a firmer basis.

Method.

Until recently our knowledge of sea water and animal fluids has been based solely on quantitative chemical analyses. F. Bottazzi[1] at Naples in 1897 calculated the osmotic pressure from the depression of the freezing point. This method has since been used by several investigators working in the same field (Rodier, Quinton, Frédéricq) and was adopted as most convenient for our purpose. Employing the Beckmann apparatus the freezing point is determined. For aqueous solutions a freezing point below that of distilled water signifies the presence of some substance in solution which is exerting an osmotic pressure. This pressure calculated for 0° C. is equal to about twelve

[1] These investigations were made at the Marine Biological Laboratory, Woods Holl, Mass. They were reported to the Biological Seminar, August 12, 1904.[10] Investigations conducted on the Pacific Coast will be reported later as they are still in progress.

atmospheres for a depression of one degree centigrade (— 1° C.). The depression of the freezing point is designated Δ. Owing to the super-cooling which takes place in solutions as strong as those with which we are dealing, inconstant results are obtained unless freezing is induced by innoculation with a tiny crystal of ice as soon as supercooling of about three tenths of a degree has taken place. A few earlier experiments in which this technique was neglected have not been recorded here. Invertebrate blood clots slowly and the first clot is easily broken up so that the freezing point of fluid as a whole may be determined. Teleost blood was whipped before freezing but no attempt was made to remove the corpuscles in as much as it has been found that they exert no appreciable effect upon the freezing point (Hamburger[11], Hedin[12]).

Osmotic Pressure of Sea Water.

The animals worked with at Woods Hole were obtained from so many different localities that it seemed advisable to determine the freezing point of the water from the same sources. The results of these determinations are given in Table I.

Table I.

	Buzzard's Bay.	Basin of the U. S. Fish Com.	Laboratory Tap.	"Eel Pond."
Maximum Δ	−1.835°	−1.84°	−1.84°	−1.82°
Minimum Δ	−1.81	−1.805	−1.82	−1.75
Average Δ	−1.818	−1.82	−1.82	−1.76
	Eleven samples.	Twenty-three samples.	Forty samples.	Eight samples.

The slight variations noted in the concentration of the different samples of sea water are not due to errors in observation for each sample of sea water was tested repeatedly and the results were checked by the use of three thermometers. The variations may be explained by the more or less land-locked condition of the bodies of water, the concentration being continually, though slightly, altered by the tides and by the continued advent of fresh water. After one extremely heavy rain the water of the laboratory tap showed considerable dilution Δ being — 1.78°. "Eel Pond" water also was diluted till the freezing point was only — 1.70°.

A glance at Table I. shows that the freezing point of the sea water at Woods Hole is on an average of 1.82° C. below zero. The osmotic pressure at a temperature of 0° C. is therefore about 22 atmospheres.

It is a noteworthy fact that the water at Woods Hole is more dilute than is that at Naples (Bottazzi [1]) where $\Delta = -2.29°$. At Arcachon also the sea water is more concentrated, Rodier [21] having determined its Δ to be $-1.89°$ C. According to our determinations made at Pacific Grove, California, the sea water of the Pacific Ocean freezes at $-1.90°$ C.

THE OSMOTIC PRESSURE OF THE BLOOD OF MARINE ANIMALS.

Invertebrates. — The body fluids (or blood) of a number of invertebrates were tested and in every case the freezing point was found to be the same as that of the sea water from which the animal was taken, no variation of more than two hundredths (0.02° C.) of a degree being found. The following list includes the forms worked with and indicates the fluid tested.

Echinodermata :

1. *Thyone briareus* — fluid from the perivisceral cavity.
2. *Arabacia punctulata* — fluid from the perivisceral cavity.
3. *Asterias vulgaris* — fluid from the perivisceral cavity.

Mollusca :

4. *Sycotypus canaliculatus* — blood obtained by section of the foot.
5. *Venus mercenaria* — blood obtained by section of the foot.
6. *Mya arenaria* — blood obtained by section of the foot.

Arthropoda :

7. *Homarus Americanus* — blood.
8. *Limulus polyphemus* — blood.

Selachians. — Two forms were worked with, the blood being obtained from the caudal artery.

TABLE II.

	Maximum Δ.	Minimum Δ.
1. *Mustelus canis*	— 1.90°	— 1.86°
2. Sand shark	2.03	— 1.88

The osmotic pressure of the blood and body fluids of *invertebrates* is due exclusively to the salts which are in solution, the proteid molecules being so large that they exert no appreciable osmotic pressure. Analyses made by L. Frédéricq[7] show that the salts in the blood of a large number of invertebrates are present in the same concentration as in the sea water. Although the blood of *selachians* has a freezing point approximately the same as that of the sea water, the salts are present in much smaller amount, 1.6 per cent. to 2.3 per cent. according to different analyses. The high osmotic pressure is maintained by the presence of a large and variable amount of urea (2–3 per cent., V. Schroeder,[24] Quinton,[20] Rodier,[22] Frédéricq[9]).

Teleosts.—The blood of all teleosts examined showed a low osmotic pressure which, in round numbers, approximated one half that of the sea water.* In Table III. are given the extreme variations in the freezing point for individuals of each species.

TABLE III.

	Source of Blood.	Maximum Δ.	Minimum Δ.
1. Sword fish	Heart after death.	—0.96°	—0.90°
2. Tautog. (*Tautoga onitis*)	Branchial artery.	—0.86	—0.86
3. Squeteague (*Cynoscion regalis*)	" "	—0.864	—0.86
4. Conger eel	" "	—0.82	—0.80
5. Common eel (*Anguilla chrysypa*)	" "	—0.90	—0.90

The results of all these investigations on marine animals agree with those of F. Bottazzi[1] and of Frédéricq[9] at Naples. These investigators found that invertebrate blood froze at —2.03° as did also that of selachians, while teleost blood showed $\Delta =$ —1.04°. The slightly greater depression of the freezing point found by these authors is to be accounted for by a greater concentration of the sea water at Naples than at Woods Hole.

VARIATIONS IN OSMOTIC PRESSURE OF THE BLOOD DUE TO CHANGES IN THE CONCENTRATION ON THE EXTERNAL MEDIUM.

The analyses of L. Frédéricq[7] showed that the concentration of the salts in the blood of invertebrates varied with the concentration

* Incidentally it was observed that the red corpuscles of teleosts were crenated by sea water.

of the salt water from which the animals were taken. This was most strikingly shown by the blood of *Carcinus mænus* taken from brackish water and from sea water. In the course of our investigations it was found that differences in the freezing point of the blood of invertebrates accompanied differences in the freezing point of the sea water from which they were taken; thus the blood of lobsters taken from the "basin" traps showed $\Delta = -1.82°$, but when taken from the "eel pond" $\Delta = -1.77°$. A decrease in the concentration of the water from the laboratory tap, due to severe rains, caused exactly the same changes in the freezing point of lobster's blood, Δ became $-1.78°$. An increase in the osmotic pressure of the blood of *Limulus* was induced by a two days' exposure to the drying influence of the atmosphere. The freezing point went down to $-1.90°$. The blood of a *Limulus* kept alive for two weeks in a damp cellar froze at $-2.03°$, the normal $\Delta = -1.82°$. Both diurnal and seasonal changes occur in the concentration of the water of San Francisco Bay, Cal. (taken near the Golden Gate), and the perivisceral fluid of starfish shows exactly the same changes; thus on March 17, 1904, $\Delta = -1.47°$ at high tide but at low tide only $-1.385°$. On September 23, $\Delta = -1.80°$.

With these facts as a starting point it was decided to test the freezing point of the blood when the animal was subjected to a large decrease or increase in the osmotic pressure of the external medium.

Dilution of the sea water was first tried and after a longer or a shorter immersion the animal was removed and the freezing point of the blood was determined. The changes which are thus induced are given in Table IV.

In nearly every experiment the animals were kept in the dilute medium until collapse set in, but in a majority of cases they were able to revive when replaced in normal sea water. *Limulus* and *Sycotypus* are particularly hardy and it is noted that the freezing point of their blood changes very quickly until in some cases it is approximately equal to that of the external medium. When the external medium is very dilute death may occur before this equalization takes place; this was particularly true in the case of *Homarus*, which is very susceptible to a change in the con-

TABLE IV.

	Sea Water Diluted with an Equal Volume of Distilled Water, $\Delta = 1.02°$.		Fresh Water, $\Delta = -0.02°$.	
	Duration of Immersion.	Blood Δ.	Duration of Immersion.	Blood Δ.
1. *Asterias*	7 hours.	$-1.14°$	12 hours.	much swollen.
2. *Sycotypus*	30 hours.	-1.07	12 hours.	$-1.23°$
			48 hours.	-0.67
3. *Nereis*	6 hours.	swollen.	3 hours.	swollen.
4. *Chætopterus*	6 hours.	swollen.	3 hours.	swollen.
5. *Limulus*	2.5 hours.	-1.43	8 hours.	-1.33
	52 hours.	-1.12	16 hours.	$-.90$
6. *Homarus*	2.5 hours.	-1.43	1 hour.	-1.63
	6 hrs. (dead).	-1.32		

centration of the external medium. In these experiments with dilute solutions it was found that when *Venus* was placed in fresh water it closed up so tightly that after two weeks' immersion the osmotic pressure of the blood was not lowered; perforation of the edges of the shell however admitted the fresh water and the osmotic pressure was lowered. A lobster placed in 3 vol. sea water + 1 vol. distilled water showed a lowering of the osmotic pressure, as indicated by $\Delta = -1.46°$. Marine flat worms on the bodies of Limuli became much swollen in the diluted sea water although they remain active for a long time.

Concentrated sea water obtained by evaporating until $\Delta = -3.80°$ was next used as the medium of immersion. The blood of *Limulus* after sixteen hours in this medium froze at $-3.79°$. The blood of *Homarus* in this doubly concentrated sea water froze at $-3.60°$ at the end of eight hours. In sea water concentrated to 0.8 its original volume the blood of *Homarus* froze at $-2.17°$ after two hours' immersion. These animals tolerate an increased osmotic pressure much better than the equivalent decrease. When the aquarium water is concentrated, complete equalization of enormous differences in the osmotic pressure between the external and internal media may take place without any marked symptoms of asthenia such as are seen when the external medium is dilute.

From the facts just sketched we see that all of the marine invertebrates which we have worked with are truly "poikilosmotic." Two factors may be at work in producing the variations

in the osmotic pressure, viz., the interchange of water, and of salts.

The entrance of water is proven by the enormous increase in weight, and the swollen appearance of those animals which have been placed in diluted sea water. This swelling has already been referred to (Table IV.) as noticeable in *Asterias* and the two worms *Nereis* and *Chætopterus*. In one experiment in which a *Limulus* was kept for sixteen hours in fresh water the animal became so swollen that the gills burst and the water of the aquarium became blue from the hæmocyanin of the exuded blood. The blood of the animals subjected to diluted media became noticeably less viscous and owing to its increased volume and the high internal pressure, was easily obtained from the animal. When subjected to concentrated sea water it was often difficult to secure sufficient blood from the lobster to make the desired determinations. That an exchange of salts also takes place, although far less rapidly than the exchange of water, is shown by the fact that when the animals are placed in distilled water, chlorides are eliminated and a copious precipitate is obtained upon the addition of silver nitrate. No quantitative chemical analyses of the aquarium water were made but in one such case an increase in the osmotic pressure was indicated by the change in the freezing point; *Limulus* was the animal experimented with and after twelve hours' immersion the freezing point of the aquarium water had been lowered from $-0.02°$ to $-0.23°$.

Quinton,[18, 19] experimenting with *Aplysia*, has also found an increase in weight when the animal is subjected to dilute sea water and a loss in weight in concentrated solutions, and he has further, by chemical analyses, found loss and gains in the amount of salts in the blood of this animal just equalling the respective gain or loss from the aquarium water. Frédéricq [3, 7, 8] made similar analyses.

There are many other proofs of the permeability of the invertebrate membranes to various salts. Loeb's [15] experiments on the rhythmic contractions of medusæ (*Gonionemus*) indicade the permeability to NaCl, $CaCl_2$, and KCl. The death of invertebrates is easily induced by acids and the salts of the heavy metals. Frédéricq [9] has placed potassium ferrocyanide and nitrates in the

aquarium water and later obtained positive tests for these substances in the blood of *Carcinus mænus*. We may conclude then that the membranes of marine invertebrates as a class are permeable both to water and to salts and act exactly "like a dialyzer membrane."

The path taken by the exchanged material is not so certainly known. Frédéricq[6] assumes that the branchial membranes of *Carcinus mænus* are the permeable structures but publishes no evidence supporting the view. Quinton[19] takes it for granted that it is the external wall which is permeable, and this seems to be true for *Aplysia*, the form with which he worked, as has been shown by Ph. Bottazzi and P. Enriquez.[2]

That the outer wall is the permeable structure of worms may be shown by a very simple experiment performed by the author on *Nereis* and *Chætopterus*. Ligatures were passed about the animals close to either end and drawn tight thus completely closing the alimentary canal; care was taken to avoid abrasion of the integument. When placed either in fresh water or dilute sea water swelling and increase in weight was obtained.

Limulus is an animal on which the permeability of the gills may be easily demonstrated. These structures are borne on the abdominal segment which may be bent ventrally to an angle of about 90°. When placed in this position and so propped up in the aquarium that only the abdomen is under the surface of the water, the mouth parts may be as much as fifteen centimeters above the surface. No water can enter the alimentary canal, nevertheless in equal parts of fresh water and sea water ($\Delta = 1.03°$), six hours sufficed to render the integument and gills tense and swollen. A freezing-point determination showed that Δ of blood had changed from $-1.82°$ to $-1.32°$. In another experiment with *Limulus* the animal was placed astride a narrow dish of fresh water and so supported that only the gills dipped beneath the surface with each rhythmic oscillation. After eight hours enough water had been absorbed to bring Δ down to $-1.41°$, the freezing point of the water had also changed from $-0.02°$ to $-0.20°$, and a copious precipitate of silver chloride was obtained. The gills of *Limulus* are permeable both to water and to salts. Metals in proteid combination, *e. g.*, copper of hæmo-

cyanin, of course do not diffuse owing to the enormous size of the molecules with which they are incorporated.

Selachians (*Mustelus canis*). — Dog fish kept in fresh water for one hour showed signs of asphyxia and were removed in a dying condition. The defibrinated blood showed a considerable decrease in osmotic pressure, Δ being changed to $-1.45°$ C. After three hours' immersion in sea water diluted with an equal volume of distilled water the blood froze at $-1.60°$ C.

These experiments demonstrate the permeability of the membranes to water. As has already been pointed out, the composition of the selachian blood is very different from sea water in its salt content, but the osmotic pressure is maintained by the presence of large quantities of urea in the blood. It is evident then that selachian membranes are semi-permeable. Little more than this can be said, for this group has not been sufficiently investigated. The same may be said of the cyclostomes. Since these animals are found in both fresh and salt waters, and some species migrate at certain seasons from salt into fresh water, the author is making a more careful study of these groups. Mosso [17] found that selachian red-blood corpuscles were laked in a 2.5 per cent. solution of sodium chloride, and that when a selachian (*Scyllium*) was placed in fresh water death resulted in a few hours. He describes a disintegration of the blood corpuscles with the formation from their débris of a sort of coagulum which plugs up the branchial arteries with consequent death from suffocation. Death resulted in one half hour if the tails had been cut off before immersion in the fresh water.

Teleosts. — In nature we have experiments of the sort under consideration, in the movements of such fish as the eels and salmon, which go from salt into fresh water at the spawning season. We have as yet no data relative to changes in osmic pressure of the blood coincident with these migrations. The author experimented with *Auguilla chrysypa*. This form lives equally well in salt water or fresh-water aquaria and tolerate sudden transmission from one medium to the other without apparent injury. In testing the freezing point of the blood in different media variations were found, but it was impossible to attribute them to the actual changes in osmotic pressure, for similar variations were found in

different tests made with animals taken from the same medium. In all these experiments it was necessary to sacrifice several animals to get even a minimum amount of blood for making determinations, so that the results were on the whole unsatisfactory; still, they suffice for the conclusion that only a slight, if any, change is induced by a change in the osmotic pressure of the external medium. The animals are "homoiosmotic."

Fundulus heteroclitus is a hardy little fish well suited to this form of experimentation, although the quantity of the blood is too small to admit of making cryoscopic determinations. It was found that if care was taken to select individuals which were not injured in catching, about eighty per cent. lived in fresh water for six weeks when the experiment was discontinued. This is as high a percentage as can be kept alive in the sea water aquaria of the laboratory. When placed in external media of concentrations varying from fresh water to sea water concentrated to twice its normal strength they showed the same hardihood. It is reasonably certain that the osmotic pressure of the blood does not change to any marked degree for an examination of the blood corpuscles did not show either laking in the dilute media or crenation in the media of higher concentration. The integument and gills are therefore impermeable. Loeb [14, 16] has found that *Fundulus* embryos will live in distilled water and in sea water to which 5 per cent. NaCl has been added.

The view that the membranes of these fish are completely permeable (Brown) [25] is not tenable, at least concerning adult *Fundulus*, as is shown by the following series of experiments which were repeated often enough to assure the verity of the results.

A large number of healthy specimens were selected and about one half the body surface denuded of scales by gentle scraping with the edge of a scalpel, or the skin was removed over an area of one square centimeter on each side; then they were divided into three lots and placed respectively into fresh water, sea water diluted with an equal volume of distilled water, and normal sea water. Of those kept in fresh water in every experiment from eighty to ninety per cent. died within twenty-four hours while all died in less than thirty-six hours. In normal sea water the fish suffered a similar fate although death did not intervene so soon.

But of those kept in sea water of one half its normal concentration only three per cent. were dead at a time when all those in the other two media had died, and seventy per cent. were kept alive for four weeks, when the wounds were all healed and the experiments discontinued. In these experiments therefore, no deleterious effects obtain when the internal and external media are approximately isotonic—in spite of the injuries and free interchange between blood and aquarium water. In the hypotonic and hypertonic solutions, however, distinct changes resulting in death, take place. In the strong solutions (normal sea water) microscopic examination showed that the blood corpuscles were crenated. In fresh water the fish became greatly swollen indicating the absorption of water. Whether laking or swelling of the corpuscles takes place was not determined in this series of experiments.*

From these experiments we may conclude that in all probability the blood of *Fundulus* does not suffer much if any change in concentration when the fish is transferred from salt water into fresh water or vice versa, provided the membranes are uninjured. If these experiments admit of general application to migratory teleosts they would indicate that these animals also are in some way protected from changes in the osmotic pressure of the blood and tissues and that the principal protective factor probably lies in a lack of permeability of their membranes. We may further conclude that in case of serious abrasion to the integument the membranes become permeable and a change of osmotic pressure of the blood results, a change which may induce the death of the animal. The great mortality of the salmon after spawning in the head waters of California's streams, is a well-known fact (Rutter[23]). Whether the generally battered condition of these fish at the spawning season bears any relation to changes in the osmotic pressure of the blood has not been investigated. It is not impossible that the actual cause of death lies in a decrease in the osmotic pressure of the blood and that the injuries are responsible for death only in so far as they permit the entrance of water and de-

*The hæmatocrit would doubtless prove a valuable aid in making experiments of this sort when it is impossible to obtain sufficient blood for freezing point determinations.

crease the osmotic pressure of the blood. In consequence of such an event we would expect nutritional changes and possibly a disintegration of the red blood corpuscles. Such a disintegration in many marine teleosts has been described though the resistance of blood corpuscles of the migratory fish seems to be very much greater than of other forms of fish, for they lose their hæmoglobin only in salt solutions containing as low as 0.3–0.4 per cent. sodium chloride (Mosso [17]).

The maintenance of an osmotic pressure lower than that of the sea water speaks for a relative impermeability of all the membranes of marine teleosts. The experiments we have described indicate an absolute impermeability of the integument. It may not be assumed that all the membranes are absolutely impermeable nor is there the same degree of impermeability in all teleosts as is indicated by the great variation in the resistance of marine teleosts to changes in the osmotic pressure of the aquarium water. Loeb's [14] experiments with *Fundulus* on the poisonous effects of sodium chloride indicate a certain degree of permeability of some surface. The poisonous action of heavy metallic salts, of acids, and alkalis indicate the same fact although many other factors must be taken into consideration among which is a possible and probable alteration of permeability due to these chemicals. A certain degree of permeability of young *Fundulus* is prettily shown by immersing them in sea water containing the merest trace of colored salts, such as salts of copper, cobalt and manganese. In these cases the solution may be almost colorless but in time the living embryos may become very deeply colored.

The facts indicate the probability of some regulative mechanism among teleosts (Höber [13]) the existence of which has not yet been demonstrated, and the nature of which cannot be affirmed.

Summary.

1. The sea water at Woods Hole, Mass., freezes at — 1.82° C., at Pacific Grove, Cal., Δ is — 1.90° C.

2. The blood or body fluid of a marine invertebrate has the same freezing point as the sea water from which it is taken, and therefore has the same osmotic pressure. This is also true of selachian blood, although the salt content is lower than that of

the sea water, the deficit of salts in the blood of this latter group is compensated by the osmotic pressure of the urea in the blood.

3. The osmotic pressure of teleost blood is about half that of the sea water ($\Delta = 0.8°$ to $- 0.96°$ C.).

4. A dilution or concentration of the aquarium water always causes an equivalent change in the blood of invertebrates, and osmotic equilibrium between "internal and external media" is established. Their membranes are completely permeable. This permeability is proven for the integument of worms and the gills of *Limulus*.

5. Dilution or concentration of the aquarium water causes a change in the same sense in the blood of selachians, but death ensues before osmotic equilibrium is established. The membranes of selachians are semi-permeable.

6. Only slight if any change takes place when teleosts (*Anguilla*) are transferred from salt to fresh water and *vice versa*. Normal *Fundulus heteroclitus* will live in water varying in osmotic pressure from that of the tap to sea water concentrated to double its strength. The membranes of teleosts are impermeable, or the fish possess some regulative mechanism which keeps the osmotic pressure of the blood nearly constant. Extensive abrasion of the skin of *Fundulus* results in death in aquarium water of less or greater osmotic pressure than that of their blood, for example they die in fresh water and in *normal* sea water but not in sea water diluted with an equal volume of distilled water.

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